CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 1 JULY, 1941 NUMBER 7

The Inheritance of Retinoblastoma and Its Relationship to Practical Eugenics*

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(Received for publication May 19, 1941)

In a brief review (51), published several years ago, the author called attention to the fact that it was no longer possible to discuss the inheritance of predisposition to the development of neoplasms if all neoplasms were considered in one group. Satisfactory evidence was available even then that the significance of intrinsic factors, the part played by genes and by extrachromosomal factors, and the extent to which mendelian laws might be applied must be investigated separately for each kind of neoplasm. In respect to the etiology of some relatively common malignant neoplasms hereditary factors seemed to play but a minor part. Squamous cell carcinoma of the lip was an example of this group. At the other extreme were found neoplasms, both benign and malignant, in connection with which the hereditary pattern was obviously of considerable importance in etiology. For instance, this had long been known to be true of neurofibromas, particularly those of the type characterized clinically as "molluscum fibrosum." Similar evidence has continued to accumulate.

Now that attention has been directed very largely toward extrinsic agents in the causation of neoplasms through progress in the investigation of chemical carcinogens and in the study of response of tissues to viruses, it appears to be timely to select a malignant neoplasm of known hereditary import for intensive study as to its familial behavior. This is done with no desire to detract from the importance of investigation of extrinsic agents, but rather to keep alive an appreciation of the significance of the intrinsic element in carcinogenesis. Retinoblastoma appears to be well suited to this purpose, since its multiple occurrence in families has led to numerous reports in medical literature which can be gathered for study. It presents other special advantages as well, as will appear in the discussion to follow.

The neoplasm under consideration has been designated by a variety of terms. In the older literature it was known as cancer of the eyeball, encephaloid of the eye, haematodes oculi, amaurotic cat's eye, and other variants of this group of names; in the early period of microscopy, as glioma retinae, sarcoma of the retina or gliosarcoma; later as glioblastoma, and still more recently as neuro-epithelioma retinae and retinoblastoma. This list is by no means complete but it will serve to illustrate the range of titles under which the case reports appear. For several reasons retinoblastoma seems to be the best term. It is now understood to designate a group of neoplasms having a common origin but not of identical histological structure; for, just as the neoplasms of the brain and cord differ among themselves in both direction and degree of differentiation, so also do the retinoblastomas (Favaloro, 12). Histological classification within the group has not as yet been utilized sufficiently to give subgroups for genetic study. We must, therefore, deal with retinoblastomas as a whole.

General Considerations

Retinoblastoma is a highly malignant neoplasm of the eyes of infants and young children which may be present at birth, is most frequently recognized during the first and second years of life, seldom appears after the sixth year and has never been known to originate in adult life. Unlike most carcinomas, therefore, it presents the distinct advantage to the geneticist of a fairly definite "end point." In evaluating the hereditary significance of carcinoma there is always a large group of individuals in regard to whom the intrinsic liability to the disease can never be known. Death at any time prior to the ultimate physiological life limit leaves unanswered the question of hereditary predisposition. This enigmatical group is very small

* The Mellon Lecture, Pittsburgh, Penn., May 8, 1941.

Presented in abridged form before The Association of American Physicians, Atlantic City, May 8, 1940; and abstract published in the Transactions of the Association of American Physicians, **55**:308-312. 1940.

indeed in respect to retinoblastoma, since it is made up only of the stillborn and of those dying in early childhood. Thus, it will be seen that retinoblastoma offers some of the advantages that pertain to the sharply limited unit characteristics employed in experimental genetics. Some exceptions to this concept, perhaps not very important because of their rarity, will be pointed out in the discussion to follow.

Course.—Retinoblastoma first grows from its area or origin in the retina into the vitreous chamber. The patient is usually too young to be aware of the resulting unilateral visual disturbance. As the mass becomes larger a parent or attendant commonly notices the peculiar opalescent light reflex from the affected eye, which was the basis for the older name of amaurotic cat's eye. If untreated, the growth, which advances both by expansion and infiltration, may rupture the eyeball anteriorly and grow out of the orbit as a fungoid hemorrhagic mass; or it may penetrate the sclera into the orbit and extend within, or along, the sheath of the optic nerve, to reach the meninges and substance of the brain. At the same time distant metastases may occur, particularly (53) to the cranial and facial bones, pia mater and brain, regional lymph nodes, parotid glands, general skeletal parts, and liver. Once extraorbital extensions or metastases have occurred, a fatal outcome is inevitable. If the growth is removed by enucleation or orbital evisceration while it is still intraocular, or if it is destroyed by roentgen ray irradiation or by radium (38), the life of the patient may be saved. Only in extremely early cases and those with the new growth located in an especially favorable position is it possible to destroy the neoplasm by irradiation without serious injury to the eye. Therefore, actual, or functional, loss of the eye is the alternative to death. The terminal stage in the child upon whom the diagnosis was made too late, or for whom treatment was refused by the parents, presents one of the most distressing situations in the entire field of neoplasia.

To the dictum that loss of the eye is the price for survival for those on whom an early diagnosis is made, there are occasional apparent exceptions. One group is made up of those who have received an incorrect diagnosis of retinoblastoma. They are examples of partial detachment of the retina, with or without retinal proliferation. This condition may occur at any age and has nothing to do with retinoblastoma, but the presence of the resulting mass in the vitreous chamber may lead to a false diagnosis. The subsequent clinical behavior will naturally appear to be highly unusual for retinoblastoma, until the error in diagnosis is recognized.

A second group of cases, which prove to be exceptions to the general rule that untreated retinoblastoma is a uniformly fatal condition, is made up of those in whom there is apparent spontaneous retrogression of the neoplasm. Naturally, the assumption of spontaneous retrogression seldom can be subjected to complete microscopical proof, since biopsy of an intraocular growth is not done. I have recently examined an otherwise untreated eye, enucleated for retinoblastoma, in which almost the entire neoplastic mass, which had filled the globe, was necrotic. Only in one very small area were there living cells, but these were sufficient to make identification of the neoplasm possible. Spontaneous retrogressive changes might have become complete in this eye. Wells (52) has commented very recently on this interesting possibility and has brought together several observations of apparent spontaneous loss of malignancy in retinoblastomas. Most of these concern the retrogression of a small growth in the second eye, after enucleation of one eye for a more advanced retinoblastoma. No further survey of the casuistical literature dealing with this phenomenon is appropriate here, but some examples of it appear in the family histories to be given later. To the extent (at present unknown) to which it may occur, it interferes with the accuracy of statistics as to the incidence of retinoblastoma by reducing the total number of cases. If spontaneous retrogression of this highly malignant neoplasm can occur, as now seems certain, it is of far greater significance as a contribution to the knowledge of the general biology of neoplasms than in its influence upon a restricted statistical analysis.

Bilateral occurrence.—Retinoblastoma is often a bilateral condition. As a rule both eyes are not involved simultaneously nor to the same degree, although both eyes frequently show neoplasm when the patient is first examined. The interval between recognition of a new growth in the first eye and in the second may vary from a few days to as much as 3 years. There are no reasons for considering the neoplasm of the second eye to be other than a new primary. Direct extension between the eyes cannot be demonstrated. So far as can be determined from many case histories, the neoplasm of the second eye develops in the same proportion of cases regardless of how early the enucleation of the first has been performed. Usually there are no evidences of general metastatic spread at the time the second neoplasm is recognized. This is significant since various other structures, particularly the cranial and facial bones, provide a congenial situation for the growth of metastatic retinoblastoma. Moreover, when neglected unilateral retinoblastoma does metastasize, there is no predilection for the contralateral eye.

The extent to which retinoblastoma has been found to be bilateral varies considerably in different series. Adam (1) found but 8.5 per cent of his cases bilateral, while Lange (22) reported 30 per cent. In his large monograph analyzing 497 collected cases, Winter-

steiner (53) found 19.1 per cent to be bilateral. The children with unilateral retinoblastoma who die before 6 or 7 years of age or who escape from medical supervision, introduce a negative error. This is probably more than balanced by the greater clinical interest attaching to bilateral involvement, which increases the probability that such cases will appear in medical literature. Institutional series, dealing with an abundant material and fortified by an adequate "follow-up" system, will give more accurate information than is now available on the proportion of cases of retinoblastoma which become bilateral.

Incidence.—Of prime importance in estimating the effect of the hereditary influence in the causation of a neoplasm is a knowledge of the incidence of that neoplasm. Multiple appearances in a particular family assume significance only when they far exceed those which might be expected under the free operation of the laws of chance. In other words, the more rare the neoplasm, the more significant does familial concentration become. Retinoblastoma is a very rare neoplasm, indeed. Berrisford (4) found that there had been 131 cases in 1,259,452 admissions to the Royal London Ophthalmological Hospital, a ratio of 1:9614 or 0.0104 per cent. Adam (1) reported the ratio in Berlin to be 1:5832 or 0.0170 per cent. Wintersteiner (53) considered the incidence of retinoblastoma to be 1:2500 patients with disease of the eye, or 0.0400 per cent. While these figures demonstrate that retinoblastoma is a rare neoplasm, they give only its incidence among those with afflictions of the eyes of such a nature as to require medical attention. Naturally its incidence in the total population is much lower. The best information available on this point is from Hemmes (17) who found, by means of circular letters, that in the years 1927, 1928, and 1929 three-fourths of the Dutch ophthalmologists saw 12 new cases of retinoblastoma. Dividing three-fourths of the living births in Holland during the same period by 12 gave an approximate incidence of 1 case of retinoblastoma in each 34,000 births of living children. This ratio seems to be in agreement with the incidence in hospital patients as given above. These figures suffice to demonstrate the rarity of retinoblastoma, which is of such a degree that it would make little difference with the application of the ratio if subsequent studies should show that the denominator is either too large or too small by several thousands. For our present purposes we accept the rate of incidence as given by Hemmes—1 example of retinoblastoma in each 34,000 living births.

Evidence for Hereditary Influence in the Etiology of Retinoblastoma

In the preceding section basic information as to the nature, course, and incidence of retinoblastoma, necessary for understanding the discussion which follows, has been presented. Most of this material will not be new to the ophthalmologist or to the pathologist dealing with ophthalmological specimens. To those whose interests must cover the entire field of neoplasia and to the nonmedical geneticist, this summary of general considerations may prove to be essential.

The evidence for an hereditary influence in the etiology of retinoblastoma will be presented in three main divisions: (a) The Significance of Bilateral Retinoblastoma; (b) Retinoblastomatous Fraternities, and (c) Retinoblastoma in Successive Generations and in Collateral Lines.

The Significance of Bilateral Retinoblastoma

It has been stated previously that retinoblastoma is bilateral in about 20 per cent of those afflicted and that there is no adequate basis for opposing the opinion that the disease in the second eye is a new primary neoplasm. In view of the general incidence of retinoblastoma (one case in 34,000 living births) the probability that two primary retinoblastomas would be the fate of a given individual is extremely slight, provided the distribution is dependent upon chance alone. That this excessively rare event will occur in the lives of some 20 per cent of all those who have retinoblastoma is unthinkable unless some other factor than chance is operating.

This question lends itself readily to mathematical treatment. The accepted incidence of retinoblastoma of 1 in 34,000 living births, means also 1 patient with retinoblastoma for each 68,000 eyes. Since $\frac{1}{5}$ of those with retinoblastoma are afflicted in both eyes, it may be considered that there are $1\frac{1}{5}$ retinoblastomatous eyes in each 68,000; or, disposing of the fraction, 6 in 340,000. Therefore, the probability that a given individual will have retinoblastoma in the right eye is $\frac{6}{340,000}$, and similarly for the left eye. Thus the probability of having retinoblastoma in one eye only is $2\left[\frac{6}{340,000}\right]=\frac{1}{28,333}$. This is simply a modification of the case incidence of $\frac{1}{34,000}$ to include the factor of bilaterality by considering eyes rather than patients. However, the probability that one individual would have retinoblastoma in both eyes is $\left[\frac{6}{340,000}\right]^2=3.114\times10^{-10}$ or approximately once in 3 million times, provided the distribution is determined by chance alone. The actual or observed incidence of bilateral retinoblastoma is approximately 1 case in 170,000 ($5\times34,000$) living births, or about 19,000 times greater than would be expected under random distribution.

If the incidence of bilateral retinoblastoma is such as to prove that this mode of appearance cannot be due to chance alone, it must be due either to *extrinsic factors* (noninherent in the germ plasm) or *intrinsic factors* (inherent in the germ plasm), or to both. These terms are not entirely synonymous with *environmental* and *hereditary,* but approximate them suffi-

ciently closely that the latter may be substituted in the present discussion. There is no information at present which in any way indicates that an *environmental* factor plays a part in the causation of retinoblastoma. Such an influence may be discovered in the future but there is every indication that in the etiology of this neoplasm extrinsic factors must play an exceedingly small part, if operating at all. This point can be discussed more effectively after illustrating the familial distribution of retinoblastoma.

If both chance and extrinsic factors can be excluded we are left with the explanation that the development of primary retinoblastoma in both eyes is due to the fact that a common hereditary pattern has made them both vulnerable to this form of unlimited and imperfectly differentiated proliferation.

It is interesting that the percentage of bilateral involvement is much higher in familial retinoblastoma (Best, 5) than in collected series of solitary or sporadic cases. This seems to indicate that there may be quantitative differences in the intensity of the intrinsic factor or complex.

Retinoblastomatous Fraternities

Familial retinoblastoma, limited to a single generation and involving several of a group of sibs, has long been recognized. This may be designated as a horizontal distribution in a family. The earlier literature of such observations was collected by Leber (23) whose table follows:

Author	Number of children in family	Number with retinoblastoma
Newton	16	10
Thompson and Knapp	14	5
Calderini	14	3
Leber	9	3
Wilson	8	8
Lerche	7	4
de Gouvèa	7	2
von Graefe	6 or 7	2
Marshall, C. D.	6	3
MacGregor	5	3
Cirincione-Calderaro	5	4
Schönemann	3	2
Valenti	3	2
Fuchs	3	2
Snell (1st case)	3	2
von Hoffmann	3	2
Snell (2nd case)	2	2
Steinhaus	2	2
Owen	2	2
	119	63

The 19 families in Leber's compilation included 119 children of whom 63 developed retinoblastoma. Obviously, in these families retinoblastoma was not distributed by chance alone, when the general incidence is 1 example in 34,000 living births.

No effort has been made to collect for this study the retinoblastomatous fraternities which have been reported since the publication by Leber. This manifestation of retinoblastoma is so generally recognized today that examples are unlikely to be recorded unless they present some unusual feature.

The family reported by Newton (31) in 1902 is such a remarkable example of fraternal retinoblastoma (also possibly collateral) that it should be reviewed here. It is shown diagrammatically on Fig. 1. There is good evidence that the father's brother had retinoblastoma, although this was not definitely established. The disease appeared in 10 of 16 children and was bilateral in 7. There was no evidence of sex linkage since 5 of 9 boys and 5 of 7 girls were affected. Of the 6 children reported as normal, 2 died when less than 12 months old and while they were still potential candidates for retinoblastoma. The chief information in respect to the children follows:

1. Male. Died at 6 weeks of bronchitis.
2. Male. Healthy at 25 years of age.
3. Female. Alive, but described as "delicate" at 23 years.
4. Male. Retinoblastoma of right eye. Enucleation at $2\frac{1}{2}$ years, followed by local recurrence and death at 3 years.
5. Female. Retinoblastoma, bilateral. Died at about 3 years of age.
6. Female. Healthy at 19 years.
7. Female. Retinoblastoma of the left eye. Died at 2 years.
8. Male. Retinoblastoma, bilateral. Died at 3 years.
9. Male. Retinoblastoma of the right eye. Enucleation at $3\frac{3}{4}$ years, followed by recurrence and death at 5 years.
10. Male. Died at 9 months of bronchitis.
11. Male. Retinoblastoma, bilateral. Died at 3 years.
12. Male. Retinoblastoma, bilateral. Died at $2\frac{1}{2}$ years.
13. Male. Healthy at 8 years.
14. Female. Retinoblastoma, bilateral. Died at 3 years.
15. Female. Retinoblastoma, bilateral. Died at 3 years.
16. Female. Retinoblastoma, bilateral. Under observation at time of report. Operation refused.

If the incidence of retinoblastoma is 1 in 34,000, the probability of 10 occurrences in a group of 16 individuals, as in the family reported by Newton is 3.874×10^{-42}, a fraction so small that a single example in all medical literature is sufficient demonstration that some factor other than chance must determine such a concentration.

A hitherto unreported retinoblastomatous sibship.— I have had the opportunity of examining several eyes from members of a family which has not been described previously.[1] Six of the 10 children developed retinoblastoma and in 4 the disease was bilateral (Fig. 1). The paternal grandfather had died of carcinoma of the stomach. The chief data on the children follow:

1. Girl. Now 19 years old. Normal vision.
2. Girl. Left eye removed for retinoblastoma when 3 years old. The remaining eye is apparently normal. Her present age is 18 years.

[1] Most of the afflicted members of this family have been patients in the University of Michigan Hospital. I am indebted to the Social Service Department of the Hospital and to Dr. L. W. Switzer of Manistee, Michigan, for details of the family history.

3. Girl. Both eyes removed for retinoblastoma before the age of 2 years, when death occurred.
4. Boy. Now 16 years old. Has no neoplasm but is practically blind in one eye due to structural changes which have been diagnosed "deterioration of the retina."
5. Girl. Both eyes removed for retinoblastoma before the age of 27 months, when death occurred.
6. Boy. Now 9 years old. Eyes apparently normal.
7. Boy. Right eye removed for retinoblastoma when 4 years old. Died 8 months later of diffuse cranial neoplasia—"head twice normal size."

bility if one states that it means less than one chance in a trillion trillion.

Retinoblastoma in monochorial twins.—The occurrence of retinoblastoma in monochorial twins is a special form of fraternal retinoblastoma which has been reported by Benedict (3). His observation met the requirements set forth by Militzer (30) in that the neoplasms were similar, simultaneous, and symmetrical. The development of such a rare neoplasm in the left eyes of both members of the pair would

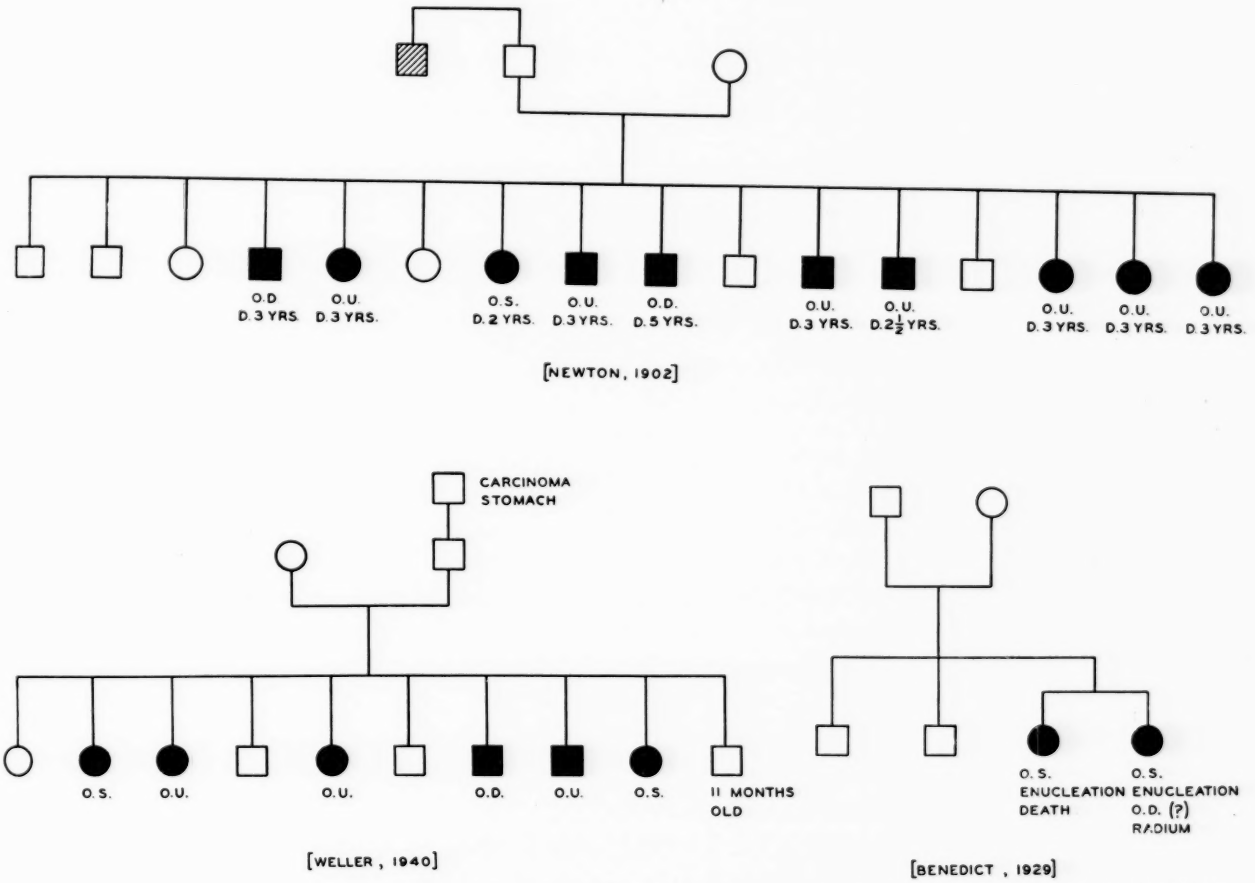

FIG. 1.—Selected retinoblastomatous fraternities, one of which has not been previously reported. (In this, and in the following figures, conventional symbols are used. Squares indicate males; circles, females; triangles, individuals whose sex was not stated, and numerals within symbols signify siblings to the number stated. Affected individuals are shown by solid black symbols. Shaded symbols indicate possible, but imperfectly established, examples of retinoblastoma. O.D., O.S., and O.U., indicate respectively, involvement of the right eye, of the left eye and of both eyes. An abstract of the available data concerning each family shown in the figures will be found in the text.)

8. Boy. Right eye removed for retinoblastoma when 16 months old. Left eye removed when 44 months old. Cranial metastases and death 3 months later.
9. Girl. Left eye enucleated at 20 months for retinoblastoma. Right eye normal at last report. (Autopsy Dec. 1943)
10. Boy. Five months old at last report. Eyes then apparently normal.

The probability that 6 examples of retinoblastoma would occur, by chance alone, in a fraternity of 10 members is 1.358×10^{-25}. It does not greatly assist in comprehending the infinitesimal degree of this probability if one states that it means less than one chance in a trillion trillion.

Retinoblastoma in monochorial twins.—The occurrence of retinoblastoma in monochorial twins is a special form of fraternal retinoblastoma which has been reported by Benedict (3). His observation met the requirements set forth by Militzer (30) in that the neoplasms were similar, simultaneous, and symmetrical. The development of such a rare neoplasm in the left eyes of both members of the pair would be difficult to explain on other than an intrinsic basis. A diagram of this family appears in Fig. 1 and brief notes on the cases follow here:

Benedict, 1929 (3). The mother of the affected twins had a twin sister, twin nieces, and 2 sets of female twin cousins. Thus, twinning, limited to females, was probably an hereditary trait in this family. The twin girls developed retinoblastomas in their left eyes at about the same time. One died 6 months after enucleation. The other survived, but developed a small growth in the right eye. This apparently retrogressed following the application of radium. Six years after the enucleation and at the time of the report, there was increasing cloudiness of the lens, but no sign of neoplasm.

Retinoblastoma in Successive Generations and in Collateral Lines

Knowledge that retinoblastoma may occur in successive generations of the same family, thus exhibiting a vertical inheritance in contrast to the horizontal type described in the preceding section, has been a relatively recent development. In 1908 von Hoffmann (49) gave what he believed to be the first report of glioma retinae in successive generations of the same family. He was in error in that belief since von Graefe (48) in 1868, Thom[p]son and Knapp (43) in 1874, de Gouvèa (10) in 1886, Marshall (28) in 1897, Steinhaus (39) in 1900, and Taylor (41) in 1905 had preceded him with reports of the occurrence of retinoblastoma in successive generations of the same family, although these examples were not all equally well authenticated. von Hoffmann's assertion of priority is a clear indication of how little knowledge there was among ophthalmologists that inheritance of this type could be demonstrated by retinoblastoma.

That there were not more examples of vertical inheritance observed during the earlier period was due to the fact that there were practically no survivors among those who developed retinoblastoma. With prompt diagnosis and early enucleation, or evisceration of the orbit, an increasing number of those who had had retinoblastoma survived to marry and to procreate. This group has been largely responsible for the many examples now available of retinoblastoma in successive generations of the same family.

In addition to the many examples of direct inheritance there have been reported other families showing transmission through an unafflicted parent or the appearance of the disease in collateral lines rather than in lineal descendents. For earlier collections of families with retinoblastoma in more than one generation, reference may be made to the monographs of Clausen (9) and of Julia Bell (2) which are important sources of data in this field. To the families which they reported have been added all of the examples of familial retinoblastoma, other than those confined to sibships, which I have been able to find. Abstracts of these follow, giving such data as are available. The arrangement of this material is not chronological, but in groups of examples which have some genetic characteristic in common.

Apparent inheritance of retinoblastoma through an affected male parent.—Fourteen families have been found in which the father of one or more affected children had had retinoblastoma. These are shown diagrammatically in Figs. 2 and 3.

de Gouvèa, 1886, 1910 (10). This family includes retinoblastoma in a father and in 2 daughters among 7 children. The right eye of the father was removed in 1872 when he was 2 years of age. He married at 21 years. The first child was normal; the second, a girl, developed bilateral retinoblastoma and died at 2 years; the third, also a girl, had bilateral retinoblastoma and died at 5 months. The sex of the 5 normal children is not given. (de Gouvèa stated that this family was reported by him in the Bull. Sociedade de Medicina e Cirurgia do Rio de Janeiro, August 25, 1886; and subsequently in the Annals of the Academia de Medicina of that city. Through these reports, not known to other early writers on familial retinoblastoma, he claimed priority in this field.)

van der Hoeve, 1926 (47). The father lost one eye (side not stated) when he was 3 years old. Of his 12 children, the sixth and the eleventh developed bilateral retinoblastomas. The sex of the children was not given in the report.

Sym, 1928 (40). The father was operated upon for glioma of the left eye at the age of 9 months. He recovered, married, and had 4 children. At 27 he died of multiple intracranial new growths, the nature of which was not given. His first child was a stillborn fetus of which the sex was not stated. The second child was a girl who died at about 4 years of age. She had bilateral retinoblastomas, and the father refused to permit operation. The third child, a boy, also had bilateral retinoblastomas and died when about 4 years old. Again the father refused operation. The fourth child, a girl, was apparently normal at 3 years. The author comments that, racially speaking, the father may have been wiser than he himself knew in declining to have the 2 affected children operated upon.

Maher, 1902 (27); Pockley, 1919 (34). Maher reported this family because of multiple examples of "glioma" in one generation. In 1902, the oldest child, a boy, was apparently normal at 8 years of age. The second child, also a boy, had had one eye removed at 18 months and the other when 2 years old. He was surviving at the age of 7 years. A daughter had had both eyes removed at 2 years and was living at 5 years. The fourth child, a girl, was living at $3\frac{1}{2}$ years, after removal of the left eye when 1 year old. Pockley reported that the father of his patient had had both eyes removed by Maher and that 2 of the father's sisters had had *both* eyes removed in infancy. Thus these two reports must refer to the same family. The affected boy survived to marry a woman who was blind in one eye as was also her mother, but there is no evidence that their blindness was due to retinoblastoma. The sex of the child from this union is not stated, nor is information available as to whether one or both eyes were involved. At least one eye was removed since the diagnosis of retinoblastoma was confirmed by microscopical sections. Our diagram utilizes information from both reports.

de Haas, 1916 (11). In 1913 de Haas enucleated the right eye of a child (sex not stated) who had bilateral gliomas. The mother would not consent to the removal of the left eye. The father of de Haas had enucleated the right eye of the father of this child in 1882 for glioma retinae.

Hemmes; Waardenburg, 1932 (50). Waardenburg credits Hemmes with the observation of bilateral retinoblastoma in a girl whose father had survived enucleation for unilateral retinoblastoma. No other data are provided.

Stallard, 1936 (38). From the father in this family the left eye was removed for glioma retinae at the age of 14 months. The nature of the process was confirmed by microscopical examination. The right eye appeared to be similarly affected, but the parents had refused to allow enucleation. At the age of 4 years, he had had a very severe attack of scarlet fever, during which the right eye gradually "cleared." In 1934, when 34 years old, there were irregular pale areas in the eyeground and some additional pigmentary disturbances. There was no other evidence of an intraocular neoplasm and no defect in visual acuity. His older son suffered enucleation of the left eye for glioma retinae when $2\frac{1}{2}$ years old. The right eye was found at that time to contain a white mass which was diagnosed clinically as glioma retinae. The parents refused enucleation of this remaining eye

which was treated with radon. The growth diminished and 2 years later there was no evidence of local spread. Vision was adequate for playing with other children. The younger son was noted to have bilateral glioma retinae soon after birth. Both eyes were nearly full of neoplasm. Enucleation was refused by the parents. He was still living, but totally blind, at the age of 27 months.

Letchworth, 1928 (25). The father of this family suffered the loss of his right eye for retinoblastoma when 2 years and 3 months old. Of his children 1 died at birth and 1 was living and well at the time of report. The sex and sequence of these 2 children were not given. Leonard, born in 1919, developed bilateral retinoblastomas which caused his death. Walter, born in 1921, had his right eye enucleated for retinoblastoma at the age of 3 months. He was otherwise well when 7 years old. Ernest, born in 1928, developed retinoblastoma of the right eye which was removed when he was but 2 months old. He was living when the family was reported.

Traquair, 1919 (45). The left eye of the father was removed for tumor when he was 6 months old. His first child was stillborn. The second, George, had his right eye removed in 1914, at the age of 6 months; and the left eye was removed in 1917. He died with generalized metastases in 1918. The third child, Mary, developed retinoblastoma of the left eye. Operation was refused and death was attributed to bronchopneumonia. (The children's names were obtained from Fietta (13).)

Griffith, 1933 (15); Hine, 1937 (18). Hine described a family which had been reported by Arthur Griffith in 1933. The grandfather and grandmother on the paternal side were free from neoplastic disease of the eyes. A son, born in 1895, suffered enucleation of the left eye when 2 years old. He was thought to have a healed lesion of the right eye. This boy survived, married, and had 5 sons. The first was prematurely born and died at the age of 6 months. The second, 19 years old at the time of report, showed areas of choroidal and retinal atrophy which Hine believed were sufficiently characteristic to justify an opinion that they represented bilateral healed gliomas. The third son had retinoblastomas of both eyes and died at 21 months. Bilateral enucleations had been done. The fourth son was living at 11 years of age. The left eye was normal. The right had been removed for retinoblastoma, first noted at 3 months. The fifth son died when 4 years old. He had developed bilateral retinoblastoma. One eye was enucleated and the other treated with radon.

Lange, 1938 (22). (First family.) In 1911 the left eye of a boy, 4 years of age, was removed for retinal glioma. No other disease of the eyes was known in his family. He married a woman whose eyes were normal as were those of her 11 siblings. Two sons were born to them, both of whom developed bilateral retinal glioma, one at 1½ years, followed by death, and the other at 4 years. This later child appears to have been living at the time of the report.

Meyer-Riemsloh, 1929 (29). The first-born son developed a neoplasm of the left eye, when 6 months old. The eye was removed and the diagnosis of retinal glioma was confirmed by microscopical examination. The father had lost his right eye in 1901, when 3 years of age, because of a glioma.

Lange, 1938 (22). (Second family.) A boy whose parents, grandparents, and 6 siblings were without disease of the eyes, developed retinal glioma of the left eye when 21 months old. The eye was enucleated and he survived to become a parent. Of his 4 children, one boy developed bilateral retinal glioma at 21 months and died. The left eye was enucleated and the right was treated by irradiation. Lange emphasized the fact that in the first generation known to be affected there was every indication that only a "sporadic" example of retinoblastoma was under observation.

Sleight (Weller), 1940. Through the cooperation of Dr. R. D. Sleight of Battle Creek, Michigan, I am able to include an additional example, hitherto unreported, of familial retinoblastoma with the diagnosis verified in two generations and family tradition indicating presence of the disease in two additional preceding generations. If these unproved occurrences can be accepted, this record exceeds by one generation any other as yet published.

Alexander M., of Austrian extraction but born in Michigan, was seen by Dr. Sleight in 1917 when he was 2½ years old and glioma of the left eye was diagnosed. Enucleation was advised but could not be done by Dr. Sleight as he was leaving immediately for military service. About 6 weeks later this eye was removed in the University Hospital at Ann Arbor and microscopical examination confirmed the diagnosis of retinoblastoma. The patient survived and became the father of 2 daughters. The older girl, Jacqueline, had her left eye enucleated for retinoblastoma in 1937, when 10 months old, by Dr. J. O. Wetzel of Lansing. She had been seen at the University Hospital where the diagnosis had been confirmed and operation advised. In July, 1938, the mother wrote the Social Service Department of the Hospital as follows: "Jacqueline had her right eye removed. Now her left eye is affected—she's blind. Youngest daughter, Sherson, 12 months old, also has glioma in her left eye—must be removed immediately." Dr. Sleight subsequently removed the left eye of the second daughter and also saw the first child in the final stage of the disease, enucleation of her second eye having been refused by the parents. Upon medical advice (Dr. Sleight) the mother's third pregnancy was interrupted and the father was sterilized. While the history was being reviewed with the family, Dr. Sleight was told that the father of Alexander (and grandfather of the girls) had had one eye removed when he was 3 years old and that *his* father also had had one eye removed in childhood. No practicable method of verifying this information could be discovered.

Apparent inheritance of retinoblastoma through an affected female parent.—Seven families have been found in which the mother of one or more affected children had had retinoblastoma. These are shown diagrammatically in Fig. 4.

Taylor, 1905 (41). Information in respect to this family is extremely meager. In discussing a paper by S. Snell (37), Taylor said that some years before he had removed a child's eye for glioma. The second eye was subsequently involved and he died. The mother of that child had had an eye removed in early life, and he believed that also had been for glioma.

Caspar, 1912 (7). The maternal grandmother in this family had a congenital angioma of the left upper lid and forehead. Her daughter's left eye was enucleated for retinoblastoma in 1892, when she was 2 years old. She survived and married. From the granddaughter the right eye was enucleated in 1911, at 16 months, and found to be filled with retinoblastoma.

Reiser, 1937 (36). The affected mother in this family had her right eye removed at the age of 1 year. Neither among her antecedents and collateral relatives, nor among those of her husband, was another example of ocular neoplasm known. Her son developed retinoblastoma of the left eye, which was removed, but he died later of metastases. A daughter was unaffected at the time of report.

Griffith, 1917 (16). The Smith family. From the mother, who was 22 years old when her first son was found to have retinoblastoma, the right eye had been removed for a growth

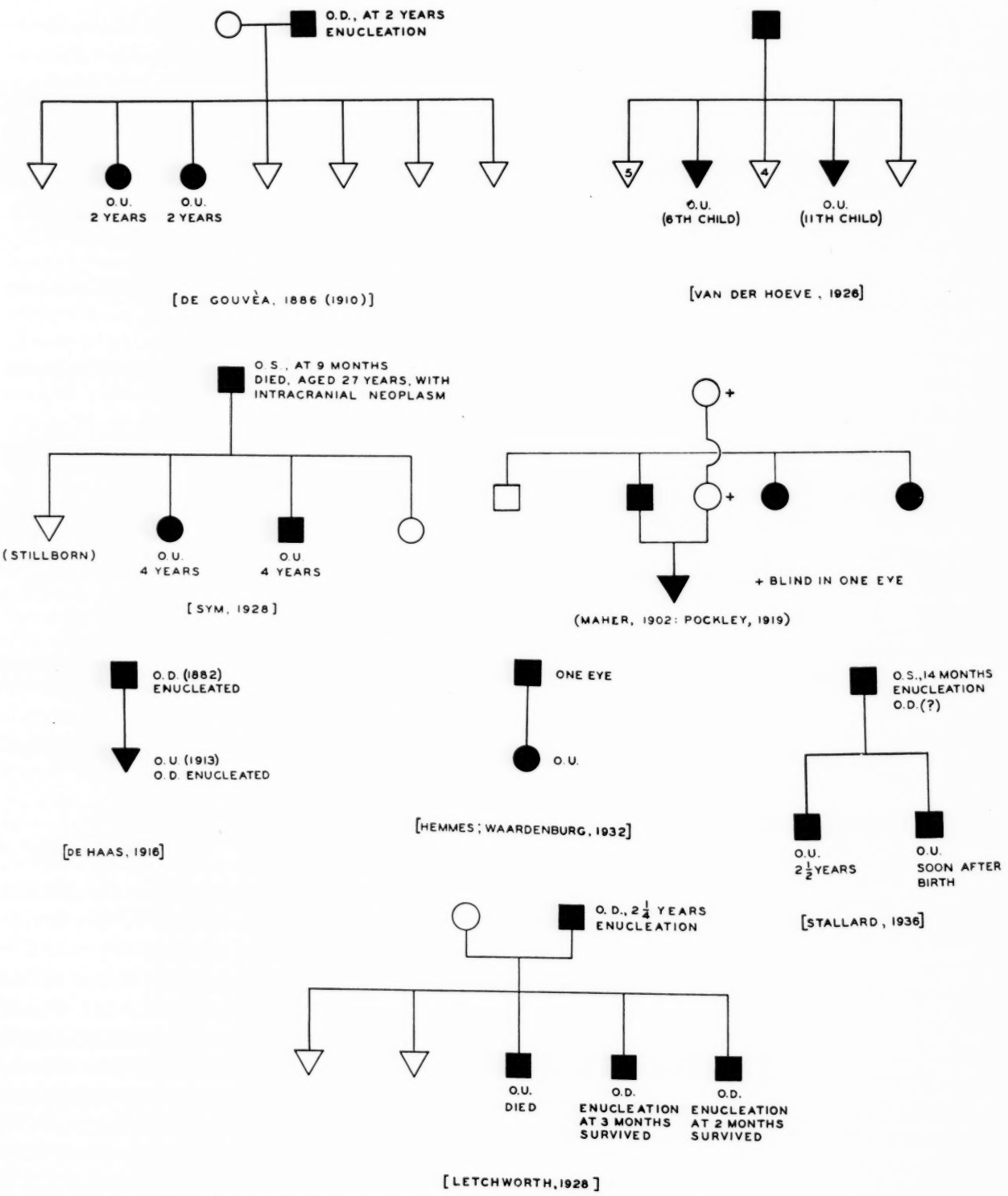

FIG. 2.—Eight families showing apparent inheritance of retinoblastoma through an affected male parent.

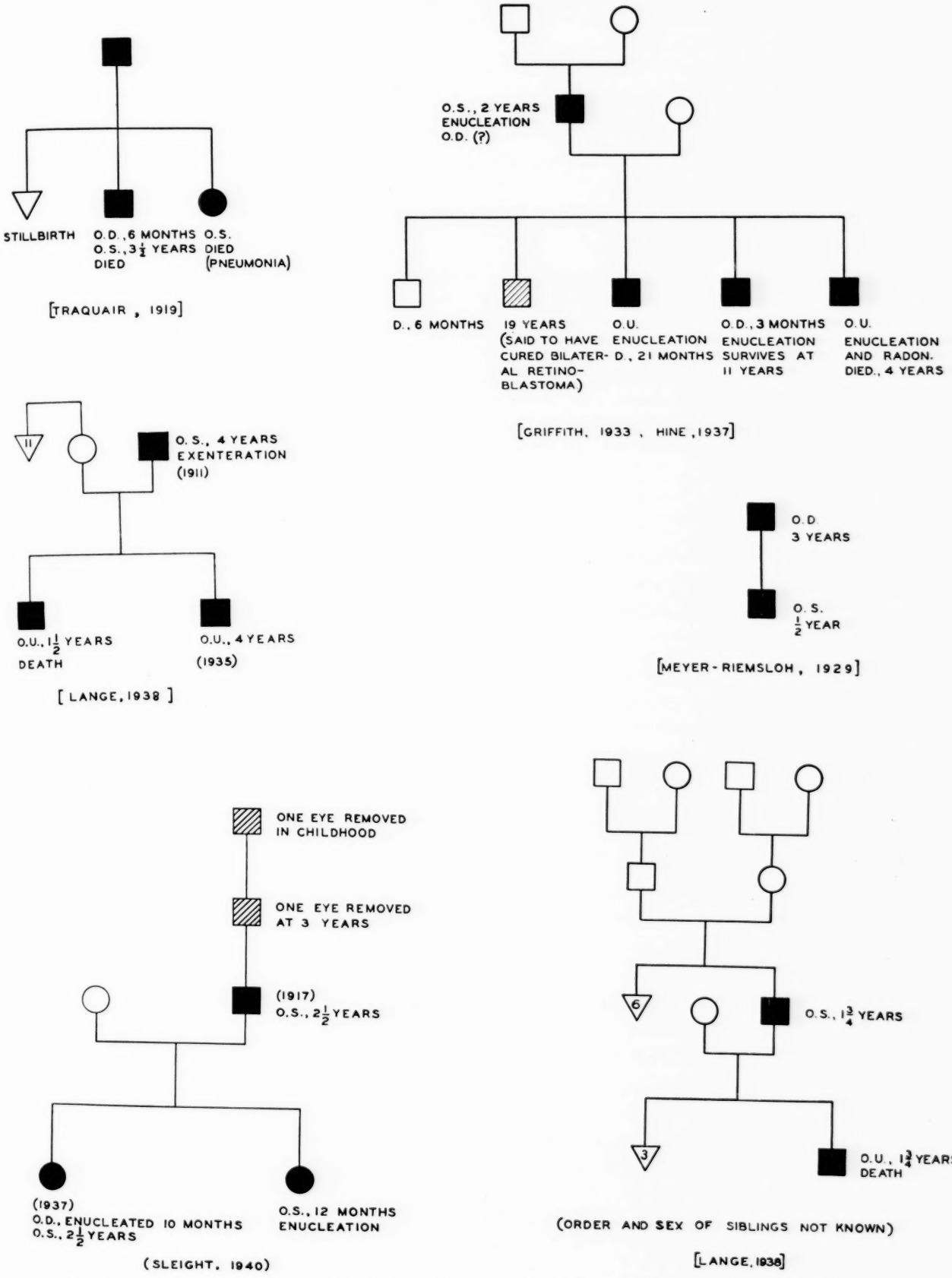

FIG. 3.—Six additional families showing apparent inheritance of retinoblastoma through an affected male parent.

at the age of 9 months. The grandmother stated that the mother's eye had been exactly like that of a grandchild and "flashed fire." The children were:

1. John. Seen January 3, 1903, when 5 months old. The left eye was removed for glioma a few days later. In September of the same year the mother found the right eye affected and it was removed shortly thereafter. This child was well until the age of 7 years, when a tumor developed in the right upper jaw and palate. He died 3 weeks later and both kidneys were found to be involved. There was no growth within the skull. (From this it is far from clear that the original neoplasms of the eyes were the cause of death.)

2. Ethel. Twelve years of age at the time of report, with both eyes normal.

3. Lucy. Growth in left eye at 6 months and in right eye at 9 months. Both eyes were removed. She died about 1 year later from measles.

4. William. Both eyes were removed for glioma and he died of this disease at the age of 2 years and 11 months.

5. This child was born dead. The sex was not stated.

6. Mary Elizabeth. Left eye was removed for glioma at 5 months. The right eye was affected shortly thereafter but was not removed. Death from bronchopneumonia at 13 months.

7. Baby, 10 weeks old at time of report. Sex not stated.

Griffith, 1917 (16). The Jones family. From the mother of this family the right eye was removed in 1879, when she was 2½ years old. Her first child was stillborn. The second, Doris, had a right-sided enucleation in 1906, when she was 3 years old. She was well at 13 years, with the left eye normal. The third child, George, also required enucleation of the right eye when 3 years old and was well at 12 years of age. In the fourth child, Florence, the disease was bilateral. The right eye was removed when 7 months old and the left at 22 months. This child was surviving at the time of the report, approximately 2 years after the second operation.

Fietta, 1925 (13). Retinoblastoma appeared in the right eyes of a mother and her daughter. From the mother, the right eye was removed at the age of 19 months. She was 36 years old at the time of report. The right eye of the daughter was removed at 22 months for the same condition.

von Hoffmann, 1908 (49); Leber, 1916 (23). When von Hoffmann reported this family he erroneously believed it to be the first account of the occurrence of glioma retinae in successive generations. His father had enucleated the right eye of the mother of his patient about 28 years before, when she was 2 years old. Her son developed bilateral retinoblastoma at 1 year. The author stated that another baby was expected and would be kept under observation. In 1916, Leber provided additional information. "Through the kind cooperation of Dr. von Hoffmann, I have received the following account: Only the right eye of the first child was enucleated and he died at the age of 4 years of cranial metastases. In the second child, also a boy, bilateral glioma retinae was recognized at the age of 4 months. Only one eye was operated upon, and this was only for palliation of pain. Death occurred in 2 years, with cranial metastases. A third child was without evidence of the disease at the age of 7 months." (Translation.)

Apparent inheritance of retinoblastoma through an affected parent, sex not stated.—There is one family which could not be placed in either of the preceding groups since the sex of the affected parent was not stated in the report. This is shown in Fig. 5.

Best, 1934 (5). Details as to sex and sequence of births are lacking. Retinoblastoma was unilateral in a patient who had 6 normal siblings. Therefore this appeared to be a sporadic case, but this individual survived and became the parent of 3 children, 2 of whom had bilateral retinoblastoma.

Apparent inheritance of retinoblastoma through a nondemonstrating male parent.—Four families have been found with retinoblastoma in more than one generation, or in collateral lines, in which the tendency to develop this neoplasm appears to have been transmitted through nondemonstrating male parents. These are shown in diagrams in Fig. 5.

Thom[p]son and Knapp, 1874 (43); Thompson, 1898 (42). These two reports clearly refer to the same family. Our diagram utilizes information from each account. Definite information is given of a family of 14 children, 7 boys and 7 girls, of whom 2 boys and 3 girls were affected by retinoblastoma. In 1874, only 2 of these 5 had been born. The order of births is not known, except for the order of the affected offspring in relation to one another. In 1874 it was stated that a cousin (on the father's side) of the 2 children then affected, had shown a similar condition in the left eye when 2 years old and had died a few months later; and further, that 2 children of the father's aunt had presented similar findings and had died between the ages of 2 and 4 years. By 1898, not only had 3 more children in the original family developed the disease, but further information about one of the collateral lines is given. The mother is quoted: "I did not know my husband's people, but an old lady, who did, told me that a great-aunt died of cancer of the breast, and that she had 3 children [who] had died of cancer of the eyes." This is rather slender hearsay evidence. If the *father's* great-aunt was meant, it does not accord with the earlier report. If the mother was thinking of her children's great-aunt, the only discrepancy lies in reporting 3 instead of 2 affected individuals in that collateral line.

Steinhaus, 1900 (39). In a sibship of 10 members, the third child, a son, had one eye removed for retinoblastoma when 2 years old (1875). After some months there was recurrence and death. The fifth child, a daughter, also had one eye removed (1877) for retinoblastoma. She survived in good health, with the other eye uninvolved, at 25 years of age at the time of report. The sixth child, also a girl, was in a late stage of unilateral retinoblastoma when operated upon at 7 years of age. There was recurrence and death soon after. Of 6 of the sibs, who did not have retinoblastoma, the sex is not stated. The first-born was a son, who did not have retinoblastoma, but his daughter had her left eye enucleated for retinoblastoma (1894) at 4 years of age. At the time of report there had been no recurrence and the right eye was normal. Her brother, when 8 months old (1898), was found to have a small subretinal retinoblastoma of the left eye, which was enucleated. There had been no recurrence at the time of report. It is not stated whether there were other children in this generation.

Hemmes, Waardenburg, 1932 (50). Waardenburg refers to Hemmes for this family but it was not mentioned in the extensive paper on hereditary diseases of the eye which Hemmes published in the preceding year. Data are meager. Four of the 7 siblings of the father were affected. Although a nondemonstrator himself, 4 of his 5 children developed retinoblastoma. Sex and sequence of birth were not given for either generation.

Lukens, 1908 (26). From the patient the left eye was enucleated at the age of 9 months and the right eye at 3 years and 4 months, both for glioma retinae. He was apparently well 1 year later. A cousin on his father's side had died 13 years before and the uncle and aunt recognized the appearance of the eye of the patient as being like that of their own child.

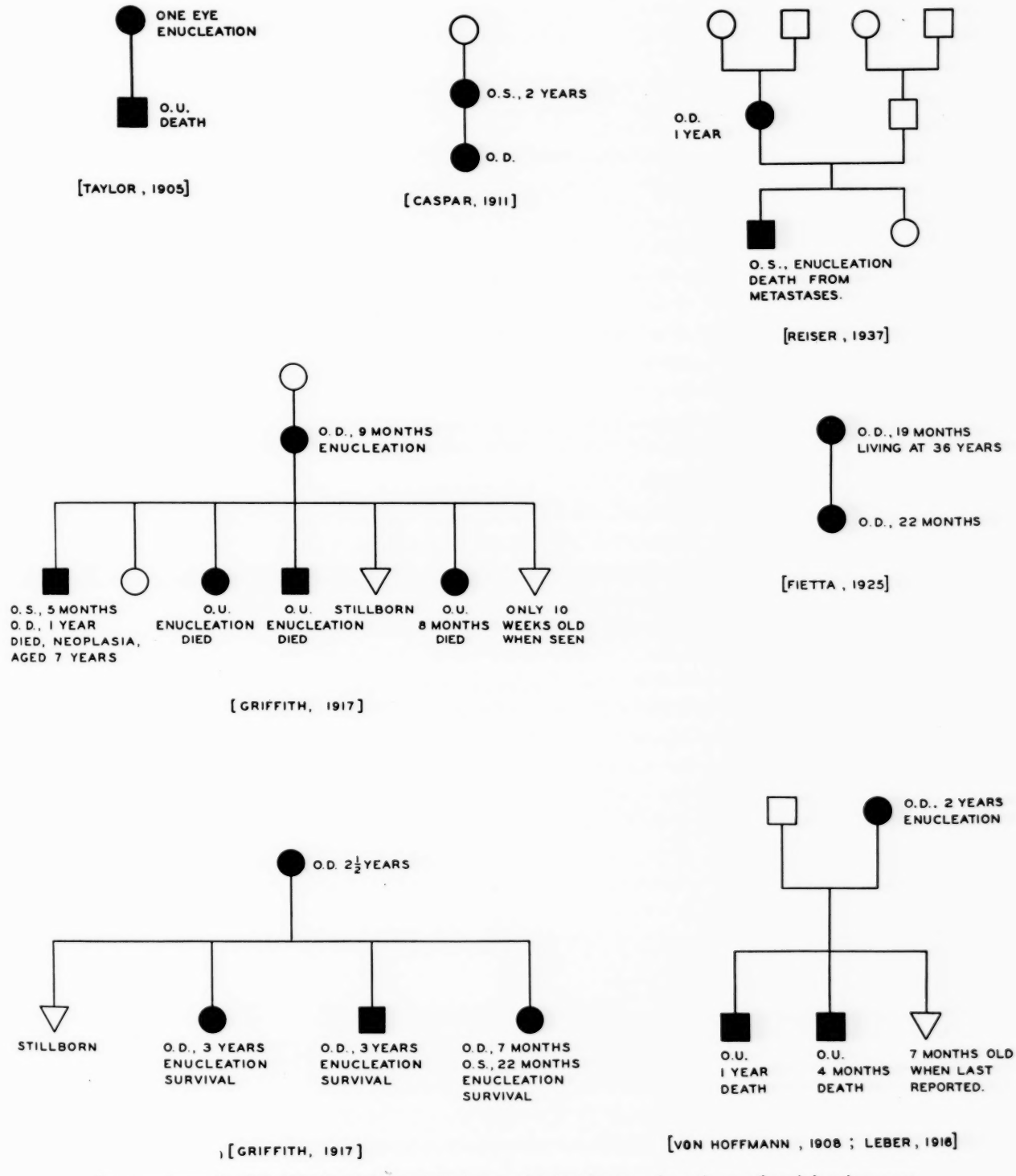

FIG. 4.—Seven families showing apparent inheritance of retinoblastoma through an affected female parent.

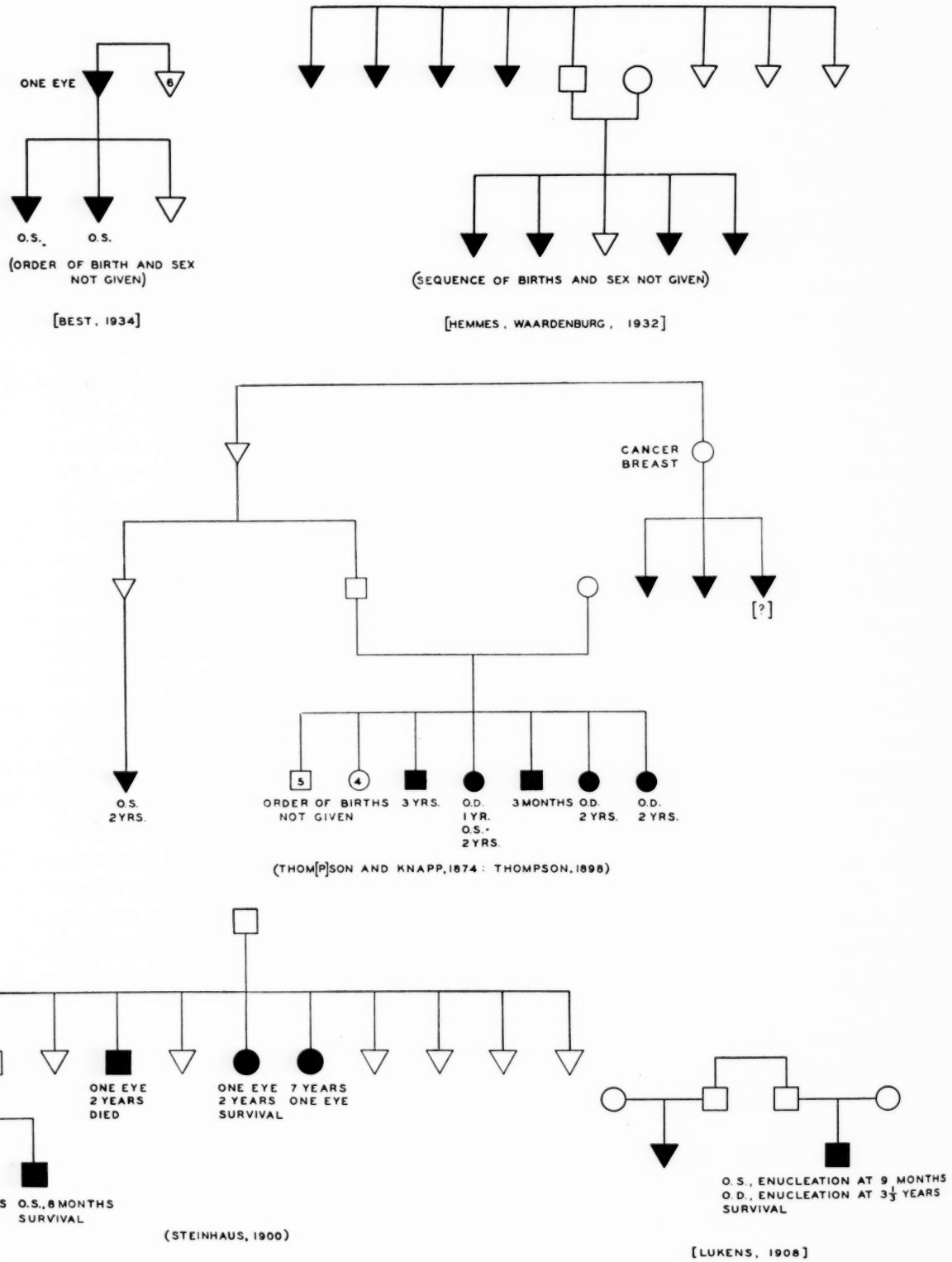

FIG. 5.—One family showing apparent inheritance of retinoblastoma through a parent, sex not stated; and 4 families showing apparent inheritance of the tendency to develop retinoblastoma through a nondemonstrating male parent.

Apparent inheritance of retinoblastoma through a nondemonstrating female parent.—Four families have been found with retinoblastoma in more than one generation, or in collateral lines, in which the tendency to develop this neoplasm appears to have been transmitted through nondemonstrating female parents. These are shown in diagrams in Fig. 6.

von Graefe, 1868 (48). Notwithstanding the meager data available, this observation should be included. After mentioning other authors who had seen several examples of glioma in one family and referring to a patient of his own with 2 siblings thus afflicted, von Graefe continued: "Several siblings of the mother of another child [with glioma] had died in the first year of life of eye-cancer." (Translation.) We know no intraocular neoplasm other than retinoblastoma which could destroy several children in the same family. This case was accepted by Bell. It is judged to be admissible to the group showing transmission through a nondemonstrating female.

Owen, 1905 (32); Berrisford, 1916 (4). The same family was described by Owen and by Berrisford. Although already a very interesting example of familial retinoblastoma on the occasion of the first report, two additional examples of the disease appeared during the following 11 years. Thomas G., the grandfather, developed a neoplasm of the left eye which was removed in 1859 when he was 5 months old. He survived and from his son, Frank, the right eye was removed for retinoblastoma in 1898, at the age of 3 years. Frank's death resulted from "paralysis" when he was 15 years old. The daughter of Thomas, Beatrice, had normal vision herself, but of her 8 children, 4 developed retinoblastoma, as follows:

Nellie: bilateral retinoblastoma; both eyes removed; died at 4 years.

Charles: bilateral retinoblastoma; both eyes removed; died at 4 years.

Julia: retinoblastoma of the right eye which was removed; died at 4 years.

Three normal daughters and 1 normal son were next in order of birth.

Gordon: bilateral retinoblastoma; died at age of $3\frac{1}{4}$ years.

There are two versions of this family in the reports utilized. The diagram in Fig. 6 and these notes follow the description of this family as given by Berrisford, but the diagram in his article and the description of the family by Owen differ in that they make Thomas G. a brother, rather than the father of Beatrice; and Frank a nephew of Beatrice, rather than her brother. With either version this family includes 7 cases of retinoblastoma and shows transmission of the trait through a demonstrating male and through a nondemonstrating female. The latter feature, as being of greater interest and possibly of greater genetic significance, determined our grouping of this family.

Townsend, 1939 (44). From the genetic standpoint this is one of the most significant reports in the literature of familial retinoblastoma. In this Negro family, through a nondemonstrating mother, the disease was transmitted, apparently, to 3 daughters by 2 marriages. The first daughter had an enucleation of the right eye for retinoblastoma at 18 months. This was done in 1933 and there was no evidence of recurrence in 1938. The second daughter, by another father, also had a retinoblastoma of the right eye when 18 months old. Enucleation was not done and multiple metastases developed in the skull, brain, facial bones, and right ovary, with death about 18 months later. The third daughter, the second by the second husband, developed retinoblastoma of the right eye in 1938, when 2 years old. Enucleation was done and this child was living at the time of reporting. The author does not state whether there were other children in addition to the 3 girls who were affected.

Purtscher, 1915 (35). This family tree of three generations includes 3 examples of retinoblastoma of the usual type and 2 other individuals with abnormal eyegrounds of such a character as to be diagnosed spontaneous retrogression of retinoblastomas. The grandfather died of sarcoma of the upper arm, recurrent in the thoracic wall following amputation. There were 11 children and 5 grandchildren, as follows (the sequence of the children is uncertain in some respects):

Ludmilla: 33 years of age at the time of reporting and married; vision normal. In this branch of the family there were 3 grandchildren, all without evidence of ocular neoplasm. The 2 boys were hypermetropic.

Karl: 32 years of age; eyes normal.

Josefa: 30 years old; unmarried; abnormalities of the eyegrounds of both eyes, but more marked in the left eye which was nearly blind. The original diagnosis was choroiditis or neoplasm, but repeated study led to the opinion of spontaneous retrogression of retinoblastomas.

Josef: 29 years old; good vision.

Thomas: traumatic death when 1 year old.

Johanna: 25 years old; unmarried; vision not good.

Ella: 24 years old; married; mother of 2 sons. The first of these, Karl, aged 5 years, had abnormalities of both eyegrounds, but more marked in the left eye with which he could count fingers only with difficulty. It was believed that these changes could best be interpreted as due to the spontaneous retrogression of retinoblastomas. Walther, the second son, developed a retinoblastoma of the left eye when $2\frac{1}{2}$ years old. This caused his death about 9 months later.

Hubert: 23 years old; good vision.

Therese: 23 years old; unmarried; very good vision.

Engelbert: developed bilateral retinoblastomas when 8 years old, which caused his death at the age of $9\frac{1}{4}$ years.

Ferdinand: found to have bilateral retinoblastomas when 10 months old. These caused his death at the age of 2 years and 8 months.

Summary of families with retinoblastoma in more than one generation or in collateral lines.—In the preceding sections there have been presented, in five categories, 30 families in which retinoblastoma has appeared as a "familial" disease, but in a pattern other than the familiar involvement of siblings alone. In the 30 families there were 102 reasonably well established examples of retinoblastoma and 6 additional doubtful cases. Before discussing the possible genetic significance of this group, attention should be turned to other evidence opposing the view that the observed incidence of retinoblastoma is indicative of an hereditary influence.

Evidence Against an Hereditary Influence in the Etiology of Retinoblastoma

There is a general clinical impression that retinoblastoma is not a familial disease. Except in large clinics, an ophthalmological surgeon will see in a lifetime but few examples of this neoplasm, and most of these will appear as isolated phenomena. Even with careful inquiry into case histories many such cases must be listed as sporadic examples. Thus, among 47 pa-

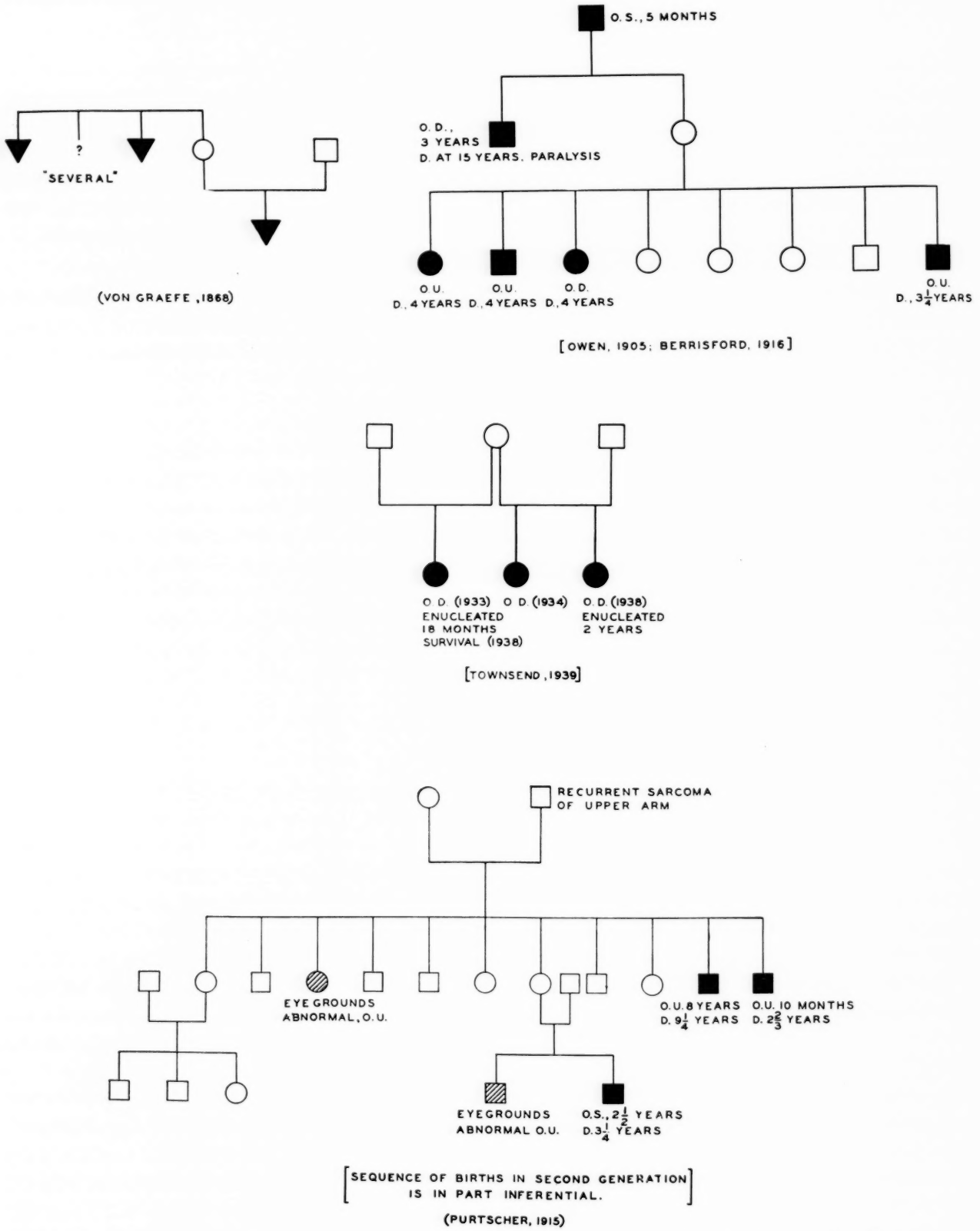

FIG. 6.—Four families showing apparent inheritance of the tendency to develop retinoblastoma through a nondemonstrating female parent.

tients with retinoblastoma, Adam (1) found no reference to the disease in ancestral or collateral line. There were, however, three pairs of affected siblings.

In the 16 families from which came the 16 cases of retinoblastoma seen in Reiser's (36) clinic in Bonn, there were 64 children, but even in large families of 7, 9, and 16 children there was but the single case. The ancestors of the 16 patients were investigated and in only one instance (Fig. 4) was a second case in the same family found. From such experiences, Reiser was led to oppose the use of retinoblastoma as an example of an inheritable neoplasm. On the average, in his opinion, about 50 per cent of those operated upon survived; and he estimated that more than 1,000 patients, the world over, had had enucleation done for this disease. Many survivors must have married and had children by this time. Yet there are but few exampies of familial retinoblastoma.

In a similar manner Keller (21) failed to find the slightest evidence of hereditary transmission in his study of 13 patients with retinoblastoma. There were many children in most of the families from which these afflicted children came. Neither among the siblings nor among the ancestors was another case of retinoblastoma found.

Hemmes (17) gathered as complete information as possible upon the families of 48 patients with retinoblastoma. Not one of the parents had had an eye enucleated. Nine cured cases of unilateral retinoblastoma were older than 25 years at the time of Hemmes' study. Two had become fathers and 2, mothers. There were 9 children in these 4 families, ranging in age from 15 months to 22 years. Not one of the 9 had shown disease of the eyes, but not all of them had passed the retinoblastoma age. The 48 glioma patients had 253 brothers and sisters, born alive. Of these, 31 died before reaching the sixth year of life and 11 were not yet 6 years old at the time of investigation. Eliminating these two groups, the 48 glioma patients had 211 sibs older than 5 years. Not one of these had had an eye removed, and not one of them had died of disease of the eye. Thirty-four of these patients came from families with more than 4 children and altogether this group had 190 sibs older than 5 years.

Further, Hemmes found that 76 sibs of 24 glioma patients had 281 children. Of these nieces and nephews, 6 were born dead, 21 died before reaching the sixth year of life and 51 were under 6 years of age at the time of investigation. None had lost an eye, and there was no evidence that any one of them was gliomatous.

The parents of 45 of Hemmes' patients were unrelated. Twice they were cousins and one of his patients was born as the result of incest between father and daughter. From these data Hemmes concluded, seemingly with good reason, that as a rule retinoblastoma appears as an isolated example and only occasionally in blood relatives, and that when retinoblastoma appears as an isolated case, it is not justifiable to forbid the later marriage of the patient.

Possible Reconciliation of Evidence Favoring and Opposing an Intrinsic Basis for Retinoblastoma

It has been shown in the preceding sections that entirely different conclusions as to a possible hereditary basis for retinoblastoma are reached, depending upon the material studied and the methods of analysis which are used. It even appears that there may be two distinct disease entities, familial retinoblastoma and sporadic retinoblastoma. However, it is apparent that these conflicting views are even now subject to partial reconciliation and it is probable that with fuller understanding of the disease they will be unified. No one can say that the victim of apparently sporadic retinoblastoma may not become the originator of familial retinoblastoma if he survives and marries. The first case to be observed in each retinoblastomatous family is of necessity considered sporadic until others appear.

Lenz (24) has shown that in view of the limited number of offspring in each human family apparent "sporadic" occurrence is precisely that which should be expected in recessive inheritance. Assuming families of 4 children each, in every 256 families in which both parents are heterozygotic there are to be expected 108 families in which only one child will show the recessive trait. All of these would appear to be sporadic cases. The families in which 2, 3, or 4 children were affected would total 67. Hence the "sporadic" cases would exceed in number the "familial" group. When the number of children per family is less than 4 the number of "sporadic" cases will be relatively greater.

Not only do we need detailed and long-continued observation of the direct and collateral descendants of the apparently sporadic case, but also a record of the ancestors of the first-recognized retinoblastomatous individual in each familial group. Such a study has been made by Lange (22) in reference to the two families charted above his name in Fig. 3. In one of these, the father of 2 children who developed bilateral retinoblastoma had lost his own left eye because of that disease when 4 years old. At that time and until his children were affected, he would have been considered an example of sporadic retinoblastoma, for no eye trouble was known in the family and 11 siblings remained normal. In the second family the father, whose left eye was removed at the age of 21 months for retinoblastoma, would have been placed in the sporadic group, for his grandparents, parents, and 6 siblings were free from this disease. Yet one of his 4 children developed bilateral retinoblastoma. Upon such evidence Lange opposed the opinion that sterilization of the surviving retinoblastomatous child is justified only if there is a family history of retinoblastoma.

In such family histories is found proof that any patient whose retinoblastoma is apparently sporadic may, with survival, become the founder of a family line containing multiple examples of this disease. It may be that only "intensity" of inheritance differentiates familial from sporadic retinoblastoma.

The Mode of Inheritance of Retinoblastoma

The collected data on familial retinoblastoma give a confused picture when the attempt is made to evaluate mendelian implications. The large proportion of affected children in the familial group (63 of 119 in Leber's (23) series) and the numerous examples of apparent direct transmission of the condition from parent to child speak for dominance and the possibility that a single hereditary factor may be concerned. On the other hand, among the families in which retinoblastoma has appeared in more than one generation and of which the charts are reproduced in this review, 15 instances appeared in which both parents were free of retinoblastoma. This suggests a recessive factor, but the evidence is far from satisfactory since consanguinity appears in but few retinoblastomatous families. Nor does it seem possible that such a recessive factor can be as widespread in the general population as would be required to give both the family reported by Lukens (26) (Fig. 5), in which the mating of 2 unaffected brothers with unrelated and unaffected women produced retinoblastomatous cousins, and that of Townsend (44) (Fig. 6), in which the mating of an unaffected female with 2 unrelated and unaffected males gave retinoblastomatous children in both instances.

It must be concluded that neither dominance nor recessiveness has been demonstrated for retinoblastoma itself. Such contradictory evidence points to some mechanism which admits of degrees of inheritance. That is, there must be inherited a basic character, probably a somatic variation, which makes retinoblastoma possible but not inevitable. Such a mechanism will provide the intrinsic potential candidates for retinoblastoma who are not demonstrators of the condition.

Others have encountered the same difficulty in attempting to formulate the mode of inheritance. Clausen, (9) in 1924, stated that he considered it very doubtful that two different hereditary mechanisms, perchance one dominant and the other recessive, are operating together in the transmission of retinoblastoma. It is more probable, he wrote, that for the development of this neoplasm some promoting or releasing factor, in addition to other elements, is essential. Actually all that he could state definitely was that in a part of the cases retinoblastoma appears to be hereditary and certainly by both direct and indirect transmission. Hemmes (17), 1931, concluded that if the basis for retinoblastoma is hereditarily dominant, not all of the parents, children, and siblings of his patients would have remained free from the disease; and that it is also improbable that it is always recessive, for in that case the required number of intermarriages between cousins and other close relatives would have been much higher than was actually the case in the population studied. Waardenburg (50), in 1932, concluded that there was evidence for both dominant and recessive inheritance in the production of retinoblastoma and that the unilateral cases suggest the mediation of some disturbing influence in the hereditary mechanism. The numerical proportion between diseased and normal children in the familial examples spoke for dominance but occasional evidences of the influence of consanguinity suggested recessiveness, although the number of such cases was much lower than the theoretical expectation. Best (5), 1934, stated that for glioma and to some extent, for other severe malformations of the eye, Mendel's laws do not apply. One must assume the concomitance of some unknown but hereditarily established derangement which can thwart normal development. Passow (33), in 1936, reproduced the conclusions of Waardenburg and stated that a final decision as to the mode of inheritance could not be given at that time but one could anticipate such information by the certainty that in part of the cases retinoblastoma was determined by heredity and by both direct and indirect transmission.

There is no evidence of sex linkage in the inheritance of the predisposition to retinoblastoma. No significant differences appear in the sex incidence among the demonstrators of the condition or among the nondemonstrating transmitters.

The failure to discover definite mendelian implications on the part of all who have investigated the inheritance of retinoblastoma is understandable if a predisposing somatic variation, itself hereditarily determined, capable of varying degrees of intensity and of continuing throughout life without developing malignancy, is assumed. Naturally, it will be very difficult to accumulate morphological evidence that such a variation exists. As early as 1897, Wintersteiner (53) stated that the occasional coincidence of glioma retinae with coloboma of the iris gave ground for the impression that congenital malformation had something to do with this neoplasm. Fuchs (14) subscribed to this view as the following quotation shows:

"The primordium for glioma is congenital in all probability. Not infrequently one finds small malformations of various sorts, indeed even very small tumors, which may be looked upon as points of origin for glioma as one studies the territory of the retina in the wider sense in the eyes of children and fetuses."

Clausen (9) summarized the earlier literature on the inheritance of various structural disturbances in the retina, including the occurrence of medullated nerve fibers, familial separation, alleged familial retinal changes associated with arthropathy, amaurotic idiocy, and angiomatosis retinae.

Inheritable retinal defects are known to occur in laboratory animals. Keeler (20) has found in the house mouse a retinal defect characterized by the absence of the visual cells (rods), absence of the external molecular layer, and reduction of the external nuclear layer to a single layer of nuclei. This defect is inherited as a simple mendelian recessive, without

influence upon viability or upon the sex ratio. All results indicated free segregation between "rodless retina" and a number of other characters which were tested. Bourne, Campbell, and Tansley (6) have described a retinal defect in rats which also is inherited as a mendelian recessive. This defect involves a marked distortion of retinal histology. The authors thought that it might have some bearing on retinitis pigmentosa.

Examples of presumptive preblastomatoid lesions, and of early retinoblastoma, in human eyes have accumulated slowly. Hirschberg (19) described multiple early gliomas as circumscribed masses of round cells in the inner nuclear layer, resembling miliary tubercles. Similar observations have been made by others but origin at other levels has likewise been described. This literature has been reviewed by Ch'in (8) who observed in the left eye of a child, whose death had been caused by retinoblastoma of the right eye, 3 small opaque nodules which could not be considered metastases. He recognized that in the early lesions of retinoblastoma "there is a certain amount of overlapping between 'malformation' and 'neoplasm.'"

Even more significant as suggesting a preblastomatoid structural deviation are the changes in the contralateral eye which, if neoplastic at all, fail to progress and are interpreted as retinitis, choroiditis, or as healed retinoblastoma. In the family reported by Purtscher (35) (Fig. 6) there was one individual (Engelbert) who had several small nodules in the partially detached retina of the left eye, with advanced retinoblastoma of the right eye. Structural changes may be found in one or both eyes of nondemonstrating members of retinoblastomatous families. This was true of 2 members of Purtscher's family (Josefa and Karl) who each had abnormalities of the eyegrounds of both eyes. The original diagnosis was choroiditis or neoplasm but further study led to the opinion that these changes were due to the spontaneous retrogression of retinoblastomas. The fourth child in a family which I have reported in this paper (Fig. 1) belongs in this group. Six of his siblings have developed retinoblastoma. He has no neoplasm but is practically blind in one eye due to structural changes which have been diagnosed "deterioration of the retina."

As a special type of glioblastoma, in the broad sense of the term, retinoblastoma should be found to be related to that group of developmental disturbances and neoplasms of the nervous system which have long been recognized as having some degree of hereditary linkage. In this group are to be found tuberous sclerosis, the glioblastomas and neuroepitheliomas proper, neurofibromatosis, 'molluscum fibrosum,' solitary neurofibromas, meningiomas, and cutaneous neurofibromatous nevi. Some of these, such as the nevi and tuberous sclerosis, have the property of remaining indefinitely in a quiescent state and then suddenly acquiring enhanced proliferative activity and rapidly becoming malignant. van der Hoeve (47) found retinal tumors in several patients with tuberous sclerosis. Some of these contained large cells resembling neurocytes, probably derived from the primitive retinal anlage. The multiple small nodular growths in the retina gave the impression of being developmental lesions rather than true neoplasms. His description of multiple round grayish white nodules in the retina is in full accord with that of the nodules seen in the contralateral eye of some patients with retinoblastoma. van der Hoeve has seen 2 patients with neurofibromatosis (Recklinghausen) who had retinal lesions like those seen with tuberous sclerosis.

Leber (23) credited Elschnig with having brought to his attention a girl of 13 years whose death was caused by an infiltrating cerebral glioma. Ten years previously her 2-year-old sister had undergone enucleation of one eye for retinoblastoma.

In reviewing the reported examples of familial retinoblastoma no reference by name to neurofibromatosis or to related conditions has been found. There are, however, rather frequent references to neoplastic conditions which might belong to this general group.

The grandmother in Caspar's (7) family (Fig. 4) had a congenital "angioma" of the left upper lid and forehead. It is possible that this was a neuromatous nevus. In the Smith family, reported by Griffith (16) (Fig. 4), a child, from whom both eyes had been removed for retinoblastoma by the end of its first year of life, died at 7 years of a tumor thought to have developed in the right upper jaw and palate. There was no growth within the skull. The right eye of Frank G., a member of the family reported by Owen (32) and Berrisford (4) (Fig. 6), was enucleated at 3 years for retinoblastoma. He died of "paralysis" at 15 years of age. The time interval is much too great to suggest metastasis from the eye. This may well have been a manifestation of a glioblastoma. In Purtscher's (35) family (Fig. 6), the father's death was caused by a recurrent sarcoma of the upper arm. One can only surmise that this may have arisen in a neurofibroma. The father in Sym's (40) family (Fig. 2), had multiple intracranial new growths when he died at the age of 27 years. His left eye had been removed for retinoblastoma when he was 9 months old. While the evidence now available does not permit statistical proof, it seems probable that such observations are of some significance as indicating a greater prevalence of such neoplasms in retinoblastomatous families than would be expected from chance distribution.

Relationship to Practical Eugenics

If it is accepted that the predisposition to retinoblastoma is determined to a considerable degree by heredity, certain practical eugenic measures would seem to be indicated:

1. Parents of a child with retinoblastoma should not have additional children.

2. Exenteration, or irradiation, if used as a life-saving procedure in a child with retinoblastoma,

should be accompanied by sterilization. Since any apparently sporadic case may be the founder of a retinoblastomatous family, this principle should be applied to the isolated example as well as to the familial case. Need for sterilization of surviving retinoblastomatous children is much greater now than it was a generation ago when few were operated upon early enough to effect a cure. Reiser (36) opposed sterilization for the sporadic case but this opinion seems not to be based on sound biological considerations.

3. The nondemonstrating members of retinoblastomatous fraternities should be advised of the possibility, by no means remote, that their children may develop retinoblastoma. Parenthood should be discouraged in this group.

Since most cases of retinoblastoma appear to be isolated examples, it may seem that the three principles stated above constitute too harsh a code; or that more evidence should be awaited before fallible human judgment should be invoked in such a manner. Those who have seen the distressing state of a child in the terminal stage of a neglected retinoblastoma, or the dismay of the parent who sees a second child similarly afflicted, will realize that a trial of such methods is indicated. Much less important considerations, offering little or nothing in the way of race betterment or the reduction of human suffering, are today frequently urged and accepted as reasons for avoiding the responsibilities of parenthood.

Summary

The extent to which heredity is important must be investigated separately for each neoplastic entity. The multiple occurrences of retinoblastoma in certain families make it a peculiarly suitable neoplasm for such a study. Although actually a very rare neoplasm (1 case in 34,000 living births), retinoblastoma is bilateral in one-fifth of those afflicted and in such patients the involvement of the second eye is to be considered an independent primary neoplasm. In many families retinoblastoma has appeared in more than one-half of the children. Such retinoblastomatous fraternities have long been known, but only recently has it been appreciated that this disease may exhibit also a vertical familial distribution, appearing in successive generations or in collateral lines. Thirty families giving evidence of this type of hereditary influence have been collected. Among these families transmission of the predisposition to retinoblastoma through both male and female, demonstrating and nondemonstrating, parents can be noted. On the other hand, a large majority of the examples of retinoblastoma, observed clinically, appear to be sporadic cases. Evidence is advanced to show the probable relationship between the sporadic and the familial group. The first case to be observed in any family appears to be sporadic.

The available data give no clear-cut evidence that the factor or factors determining predisposition to retinoblastoma behave as does either a simple mendelian dominant or recessive. This clouded picture, together with various other considerations, leads to the belief that a somatic variation, probably a structural anomaly of the retina, is inherited, and that upon this basis retinoblastoma may, or may not, develop. Hereditary retinal anomalies are known for rats and mice and have been observed in the opposite eye in patients with retinoblastoma. They have been seen also in the eyes of patients with tuberous sclerosis and with neurofibromatosis. Retinoblastoma apparently belongs in the same group with these last-mentioned diseases. That such conditions have some hereditary basis and take origin in lesions intermediate between developmental disturbances and true neoplasms is generally accepted.

Having in mind the possibility of racial betterment and the prevention of human suffering, sterilization of any child who survives enucleation or irradiation for retinoblastoma and the interdiction of further progeny to the parents of a child with retinoblastoma appear to be justifiable measures.

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Early Cancer of the Stomach*

A Study of 1,299 Resected Ulcers and 2,408 Cancers

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What constitutes an early cancer of the stomach may be open to question and may vary with different observers. It has long been known that postoperative longevity is in indirect relation to size of the primary lesion and the amount of lymph nodal involvement although there are some exceptions to this.

In order to see whether we are actually making progress in early diagnosis and treatment I began a series of observations 23 years ago. Every resected gastric cancer was measured and notes made of the lymph nodal involvement. Table I shows the yearly data.

It will be seen that there is no great difference from year to year. This may be due to the fact that by 1918 x-ray examination of the stomach had passed through the experimental stage and reached its approximate peak of efficiency. The only improvement that might be expected would be from physicians sending their patients earlier to the roentgenologist and an increase in the number of competent roentgenologists.

It was thought that some suggestion of improvement might be seen in a comparative study of the first 10 years, the last 10 years, and the last 5 years. The results may be seen in Table II.

TABLE II: COMPARATIVE STUDIES OF CANCER OF THE STOMACH (1,996 SPECIMENS)

	Average size in cm.	Percentage with lymph nodal involvement	Percentage 2.5 cm. in diameter and under
Average for 23 years	6	62	9.7
First 10 years	6.16	55.7	8.9
Last 10 years	6.2	54.6	9.32
First 5 years	6.4	59.8	6.4
Last 5 years	6	57.6	13.4

CONCLUSIONS

In the presence of these data and being personally close to all clinical facts pertaining to the patients I believe the improvement shows that general physicians are beginning to refer a greater number of patients to roentgenologists; this is certainly responsible for the improvement in our own statistics.

I believe that all medical societies should make some more extensive provision for educating the busy practitioner and his patients to secure more frequent x-ray studies in all vague abdominal distress.

* Read at the 34th Annual Meeting, American Association for Cancer Research, Inc., Chicago, Illinois, April 15, 1941.

TABLE I: YEARLY DATA IN 2,408 RESECTED GASTRIC CANCERS

Years	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	Total and average
Number	72	109	122	78	128	117	98	102	121	135	132	136	110	108	64	61	99	83	98	101	111	114	109	2,408
Percentage with lymph nodal involvement	63	65	61	51	59	51	56	59	47	45	53	41	43	56	57	45	54	46	51	58	58	50	71	62
Size in cm. with lymph nodal involvement	6.7	7.6	6.7	6.7	6.9	6.6	7	6.7	6.7	6.2	6.7	7.1	7.4	7.5	6.5	6.9	7.5	7.4	6.7	6.8	7.3	6.1	6.9	6.8
Size without lymph nodal involvement	4.7	7.1	6.9	5.7	5.5	5	5.3	4.9	5.2	5.7	5.4	4.9	4.3	6.1	4.8	5.3	6.4	6.2	4.8	6.1	5.7	4.9	4.4	4.9
Average size of all cancers	5.7	7.3	6.8	6.2	6.2	5.8	6	5.8	5.9	5.9	6	6	5.8	6.8	5.6	6.1	6.9	6.8	5.8	6.4	6.5	5.5	6	6
Largest with lymph nodal involvement	14	15	15	12	19	13	13	13	13	12	13	16	15	15	12	14	14	15	12	14	12	15	14	19
Smallest with lymph nodal involvement	2	1	2	1.5	3	1.2	2.5	1	1	2	2	2	1.8	2.5	2	3	2	1.5	1.5	0.5	2	2	1.5	1
Largest without lymph nodal involvement	11	14	12	11	13	11	10	11	12	12	13	12	9	12	9	10	14	11	12	15	11	15	10	15
Smallest without lymph nodal involvement	1.5	1.5	1.7	1	1.5	1	2	1.5	1	1.5	1	0.5	1.5	2	1	1	1.5	1	1.3	1	0.8	0.5	1.5	0.5
Percentage under 2.5 cm. in diameter with lymph nodal involvement	2	2.7	3.7	3.1	0	5.1	0	4.3	6.2	4	3.1	2.5	1.5	0	0.5	0	1.8	7	4	8	3.6	6.8	6.2	5
Percentage under 2.5 cm. in diameter without lymph nodal involvement	17.7	10	3.1	8.3	5.5	14	12	4	13	8.3	17	9	16.6	9.5	18	12	11	6.8	39	13	17	23	25	18
Percentage of all cancers under 2.5 cm. in diameter	6.1	4.7	5.5	5.3	1.3	11	5.8	4.2	9.8	5.8	8.9	5.5	7.8	4.8	10	6.6	6	7	21	10	9	14	17	12
Percentage of all cancers 2.5 cm. in diameter or larger	93.9	95.3	94.5	94.7	98.7	89	94.2	95.8	90.2	94.2	91.1	94.5	92.2	95.2	90	93.4	94	93	79	90	91	86	82	87

Malignant Melanoma Cutis

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In cancer of the skin and lips there is, from a histological standpoint, a variety of ectodermal and mesodermal tumors. Among these, melanoma is distinct because of its high malignancy. The reason for the exceptionally high malignancy is unknown. According to Adair (1) many clinically benign-looking pigmented moles excised at random are found upon histological examination to be malignant melanomas. Such tumors remain dormant unless activated by chance or by some accident. The activation occurs infrequently, since the average person has numerous nevi but active melanomas are rare. How frequently the latter occur within the population is unknown. It is not definitely established which type of nevus is more likely to be transformed into melanoma, the more common flat one, brown or dark blue, or the hairy papillomatous nevus (17). It is assumed that the transformation is due to mechanical or chemical irritation. Ewing (6) agrees that sometimes a single trauma may change a quiescent nevus into a malignant tumor. It is unknown whether or not actinic conditions which are so important in the pathogenesis of skin and lip epithelioma also play a role in the history of melanoma. Affleck (2) believes the solar stimulation of melanoblasts to be the reason for the high frequency of nevi on the head and on the hands. The activation of a nevus may occur without any local observable manifestation of the change, metastases being the first symptom. There are no histological criteria for the prediction of the course of a case (16). Neither are there definite criteria of histology or cell culture for a decision whether, with regard to the histogenesis of the nevus, Unna's epithelial theory or Soldan-Masson's theory of the neural origin is correct (7).

To add an epidemiological basis to the discussion, there will be offered a statistical analysis of 1,047 patients with skin and lip cancer. The material was collected during our former studies on surface cancer (11, 14) and on multiple cancer (10). Only that part of the material has been used here in which numerical relations could be established between all observed cases of melanoma cutis and all other primary malignancies of the skin and lip registered in the same population groups at the same time. Thus, there are included in this study all malignancies which occurred in active soldiers (1927-37) and sailors (1929-36), all cases observed during a certain period of time in the Department of Radiation Therapy of the Bellevue Hospital, New York City, and all cases collected in the Record Rooms of the Brooklyn Cancer Institute, of the Johns Hopkins Hospital in Baltimore, and in the St. Elizabeth Hospital in Washington, D. C.

Ratio and rate of melanoma in men and women.—In the 1,047 cases of skin and lip cancer, 43 patients had a malignant melanoma; *i.e.*, 4.1 per cent. In the active personnel of the armed forces this ratio was higher. Here, 20 out of 287 cases of surface malignancy were melanomas, giving a proportion of 6.9 ± 1.4 per cent. In the group of civilians, 23 out of 760 cases were melanomas; *i.e.*, 3.0 ± 0.6 per cent; for women this ratio was 4.9 ± 1.5 per cent (10 out of 204 cases), for men 2.35 ± 0.6 per cent (13 out of 556 cases). The latter figure is one-third of the figure given above for soldiers and sailors. This is a statistically significant difference. However, the age distribution of the two groups of patients is a factor influencing this difference.

In the armed forces of the United States, the 20 cases of melanoma occurred during a total of 2,433,000 person-years, the crude melanoma rate being 0.82 per 100,000. Unfortunately, the corresponding rate for the general population is unknown.

The age factor.—The proportion of melanoma to all malignancies of the skin and lip depends very much on age. The ratio is considerably higher in younger patients than in older ones (see right column of Table I), dropping from 12.1 per cent to 1.8 per cent. This decline occurs in sailors and soldiers as well as in the civil population.

Similarly, the proportion of melanoma cutis to the malignancies of all organs diminishes with increasing age. Among active sailors and soldiers, in the age group below 35 years, of all malignancy cases 4.36 ± 1.25 per cent were melanoma cutis; in the 35 to 44 year group, the ratio was smaller; *i.e.*, 2.65 ± 1.08 per cent, and of all 45 to 64-year-old cancer patients, only 0.63 ± 0.44 per cent suffered from melanoma of the skin or lip. The corresponding figures for the civil population cannot be calculated, since in civil hospitals the distribution of cancers according to age and organ is not representative for the population.

Previously, Peller and Stephenson (11) showed that in the personnel of the United States Navy the proportion of fatal surface cancers to all cancer deaths diminishes with increasing age. At this time no distinction had been made between epithelioma and melanoma. With regard to the younger age groups the reason for the high ratio of lip and skin cancer deaths can be ascribed largely to the relatively high frequency of melanomas.

The Climate.—Climatic, especially actinic, conditions appear to have an important bearing upon the organ in which, and age at which, the primary tumor appears (8, 11, 13). In white soldiers and sailors melanoma is more frequent in the southern and less frequent in the northern group.

The tendency of pigmented moles to become malignant does not increase with ageing as much as does that of other tissues, although the moles increase in number as well as in size and palpability. Obviously, there are great individual differences in the ability of moles to react to relevant factors. However, moles which have this ability respond more rapidly than other surface or internal tissues to the relevant extrinsic factors.

The site of epithelioma and of melanoma in men and women.—The distribution of melanoma on the

TABLE I: CASES OF MALIGNANCY

Age groups	A. Active personnel, U. S. armed forces			B. Four civil hospitals		Melanoma in per cent of all surface malignancies		
	Surface and internal cancer	Skin and lip		Skin and lip		In A per cent	In B per cent	In A and B per cent
		Epithelioma	Melanoma	Epithelioma	Melanoma			
—34	275	87	12	... ‡	.. ‡	12.1	...	12.1
35–44	227	84	6	... ‡	.. ‡	6.6	...	6.6
45–64	318	96	2	397	17	2.0	4.1	3.8
65+	...	..	..	340	6	...	1.8	1.8
	820 *	267 †	20	737 §	23	6.9	3.0	4.1

* Includes 56 colored patients.
† Includes 3 colored patients.
‡ These age groups were not included in my previous study, therefore they are not available here. The low age limit was chosen as 50 years of age.
§ Includes 13 negro patients.

TABLE II: STATISTICS OF CANCER IN THE ACTIVE PERSONNEL OF THE ARMED FORCES

Type of cancer	White						Colored
	Southern-born		Northern-born		Southern-born	Northern-born	
	18-34 years	35-64 years	18-34 years	35-64 years	18-64 years		18-64 years
Epithelioma of skin and lips *	63	93	24	84	156	108	3
Melanoma cutis	8	2	4	6	10	10	0
Cancer of internal organs	62	105	94	219	167	313	53
All malignancies	133	200	122	309	333	431	56

* Including the few cases of fibrosarcoma and liposarcoma.

there are 1.5 times as many southern born patients with skin and lip epithelioma as northern born, while there are only half as many southern born individuals with internal cancer as those born north of 40° latitude. The exposure to dermotropic actinic conditions during childhood and adolescence proved at least as important as the exposure during manhood. With regard to melanoma, the number of northern born patients is equal to the southern born. However, in both geographical groups, melanoma patients were much younger than patients in the other two groups of cancer cases (Table II). Comparing the number of epitheliomas with melanomas, in the southern group melanomas are less frequent than in the northern group. In comparison with internal cancer cases, surface of the body most probably does not follow the dissemination of benign nevi; neither does it follow that of epithelioma. Of all skin and lip epitheliomas in 198 women, 77.3 ± 2.9 per cent were located on the head and neck; in 539 male civilian cases the corresponding figure was 84.6 ± 1.5 per cent, and in 267 soldiers and sailors 91.7 ± 2.5 per cent.

The strong inflammatory reaction of lips to ultraviolet rays is well known to persons exposing themselves to irradiation on high mountains, on glaciers, and on snow (skiing).

Also the preponderance of outdoor workers and of rural population among cases of lip cancer has been known for a long time (15). Nevertheless, the relationship between solar radiation and epithelioma of

the lip is still underestimated (4). The chief reason for this is the low frequency of lip cancer in women, and the coexistence of other relationships in men (smokers' cancer). In Table III, of the surface epitheliomas on head and neck, 3.3 per cent originated on the lower lip in women, while in male civilians this figure was as high as 29.4 per cent, and in soldiers and sailors 40.4 per cent.

The low incidence of epithelioma of the lip in women may find its explanation in the sex disposition, in the care and protection of the lip, and in the still insufficiently studied interrelation of various organs with regard to the development of the primary tumor.

Considering only skin epitheliomas, in women 74.7 $\pm$3.1 per cent were located on the head or neck, whereas the corresponding figure in civilian males was 79.5 $\pm$ 1.7 per cent, and for soldiers and sailors 86.9 $\pm$ 2.6 per cent. It appears that the more intense the exposure to solar radiation and the less the face (including lip) and neck have been protected a) the higher becomes the incidence of surface epitheliomas, b) the higher also becomes the proportion of epitheliomas on the uncovered and unprotected parts to all other parts of the surface (Table III), and c) the higher becomes the proportion of epitheliomas on the lower lip to all epitheliomas on the surface of the head.

Table III: Number of Patients with Surface Epitheliomas

Anatomical site		Active soldiers and sailors		Four civil hospitals			
				Epithelioma *		Melanoma	
		Epithelioma *	Melanoma	Males	Females	Males	Females
Head	Skin	134	2	302	144	3	1
	Lip	99	..	134	5	..	..
		233	2	436	149	3	1
Neck		12	4	20	4	..	..
Trunk and genitals		11	7	42	30	5	2
Upper extremities		8	1	30	7	2	2
Lower extremities		3	6	11	8	3	5 †
		267	20	539	198	13	10 †

* Including the few cases of fibrosarcoma and liposarcoma.
† Among them one case, referred to later, without definitely established location of the primary tumor.

Of the 238 cases of cancer of the lip none was either a melanoma itself or combined with a melanoma originating elsewhere.

The problem of race and melanoma cannot be discussed here since the total number of colored persons suffering from a surface malignancy—7 males and 9 females—referred to in this paper is small. Cancer of the skin and lip is much less frequent among Negroes, Filipinos, and Puerto Ricans than among whites.

Of the 43 cases of primary melanoma, only 6 (13.9 per cent) were located on the head, and 23.3 per cent either on the head or neck. In men, most of the melanomas were located on the trunk, the shoulders being more frequently the site of origin than the axilla, the nipples, the inguinal, or perianal regions, respectively. In women, legs and and feet seem either to be strongly predilected, or less protected than other parts of the body.

Of 589 civilians suffering from a surface malignancy on the head, 4 (0.68 $\pm$ 0.34 per cent) had a malignant melanoma. The corresponding figures for sailors and soldiers were 235 epitheliomas and 2 melanomas (0.85 $\pm$ 0.60 per cent). This small difference becomes more pronounced if only the skin is considered and the sexes looked upon separately. Of 100 malignancies of the skin on the head, in women 0.70 $\pm$ 0.69 per cent, in male civilians 0.99 $\pm$ 0.57 per cent, and in sailors and soldiers 1.49 $\pm$ 1.0 per cent, were melanomas.

Out of 171 civilians suffering from skin cancer on the neck, the trunk, or on the extremities, 19 cases (11.1 $\pm$ 2.4 per cent) suffered from melanoma. The corresponding figures in the armed forces were 52 and 18 (34.6 $\pm$ 6.6 per cent). The difference between these two crude values (11.1 and 34.6 per cent) is statistically highly significant. Further studies will have to clarify whether this difference is not interfered with by the age factor.

Autopsy reports.—The speedy course of melanoma is due to rapid and wide dissemination of secondary deposits. Widely spread metastases may be the first clinical symptoms of the disease, although they may also develop as late as sixteen (12) or twenty-four (6) years after the destruction of the primary tumor. The average duration of the fatal cases with known data was 23.6 months with a range from 9 to 48 months. A few examples may show the spread of metastases. In the great variety of other cancers, of carcinoma as well as of sarcoma, there are not many which could compete with melanoma in the capacity of metastasizing by lymphatics as well as by blood vessels.

Wm. W. H., 22 years old. January, 1927, removal of a mole from the mastoid region. Half a year later a gland excised from the same region; histologically melanocarcino-sarcoma. Three months later, October, 1927, death. Post-mortem revealed metastases in skin, in axillary, bronchial, mediastinal, inguinal and perineal lymph nodes; in ribs, pleura, lungs, pericardium,

heart, diaphragm, liver, spleen, kidneys, small and large intestines, pancreas, and in the brain.

M. A. Sh., 25 years old. Nevus on left thigh, since birth. April, 1932, signs of activity. X-ray treatment. Half a year later excision of the nevus. February, 1933, inguinal metastasis. August, 1933, death. Post-mortem: no local recurrence; metastases to the heart, lungs, liver, spleen, adrenals, urinary bladder, and abdominal lymph nodes.

J. W. W., 37 years old. A semipedunculated mole over the greater trochanter became inflamed. Several months later, July, 1930, excision. Histologically, actively growing nevus, some variation in the size of cells, no invasion of the underlying tissue, the lesion appearing rather benign. In May, 1931, excision of a metastatic inguinal node; in October, 1932, metastases to the lungs; July, 1933, death. Post-mortem examination: metastases to inguinal, axillary, peribronchial and peritracheal lymph nodes; to intestines, stomach, kidney, liver, spleen, lungs, right biceps, left pectoralis major, and to the brain.

C. D., 51 years old. May, 1931, excision of a mole from left breast; histologically melanosarcoma. Patient died in January, 1933. Post-mortem: metastases to internal mammary, cervical, mediastinal, peribronchial, mesenteric, and retroperitoneal lymph nodes; to both lungs, pleura, heart, peritoneum, omentum, liver, adrenal, pancreas, kidney, gall bladder, intestines, urinary bladder, left testicle, temporal muscle, skin, skull, dura, and brain.

It is amazing how frequently metastases of melanoma are found in the brain. In this regard only tumors of the adrenals, of the prostate, and lungs may compete with malignant melanoma (12).

There is little doubt that the site of origin of the tumor, the intrusion of tumor cells into lymph and/or blood vessels, and their transplantation by lymph or blood are not the only conditions which decide the abundance and location of metastases. More important is the ability of the isolated and disseminated cells to survive as nests dormant for weeks, months, years, or even more than two decades and to overcome the powers hindering their aggressive growth. No approach to this basic problem has been made as yet.

Discussion

In the pathogenesis of epithelioma of the skin and lips, solar radiation is of utmost importance. Whether this is also true with regard to melanoma is unknown. Under the prevailing conditions the ultraviolet sunrays act chiefly upon the head, neck, hands, and distal parts of arms. With respect to the occurrence of cancer, the dermotropic action of the sun is followed by a considerable decline in the frequency of internal cancers. This result is but one example of a general rule, concerning the tumor-displacing function of irritation in human cancer (5, 9). According to Peller and Stephenson (11), following the skin or lip cancer a strong protection remains against the development of internal primary tumors. This has been denied by Warren and Gates (18). However, their own material seems to justify our opinion.[1] Whether also the skin itself utilizes this protection is still uncertain since the development of multiple skin and lip epithelioma at different times is not infrequent. However, the combination of epithelioma and melanoma seems rare. In only one of the 1,047 cases of surface cancer referred to in this investigation might there have been such a combination. This is a doubtful one.

H. C. H. At the age of 30, in 1894, she developed an ulcer on the bridge of the nose, which persisted until 1904. At the age of 68, in 1932, diabetes, amputation of great left toe, due to osteomyelitis, and shortly thereafter of the leg, due to arteriosclerotic gangrene. In 1934, a gland tumor in the left groin developed; biopsy proved carcinoma. In 1936, sudden death. Post-mortem examination revealed melanotic carcinoma of unknown origin with metastases in inguinal, pelvic, retroperitoneal, and cervical lymph nodes; in lungs, liver, and brain. Embolus of left main branch of pulmonary artery from thrombus in left femoral. Perhaps a clinically unsuspected pigmented mole located on the amputated leg was the primary tumor.

Since the combination of epithelioma and melanoma is rare, and the distribution of melanoma over the surface of the body so different from the distribution of epithelioma, it is improbable that the ultraviolet portion of the solar spectrum is related as a major factor to the pathogenesis of melanoma. Whatever the pathogenetic factors of melanoma are—traumas and inflammations, etc.—they manifest themselves more frequently on regions less or not at all exposed to sun, and they are, on the average, effective at an earlier age than those correlated with epithelioma. The occurrence at an earlier age may be due to the pre-existent specific tissue, the nevus, which is ready for activation or it may result because protection is later afforded to the skin by the epithelioma. The exploration of this point may prove useful with regard to Apperly's recently advanced hypothesis (3) of immunization against internal cancer by ultraviolet rays in subepithelioma doses.

Summary and Conclusions

1. Out of 1,047 patients with cancer of the skin and lip, 4.1 per cent had malignant melanoma cutis.

2. In the civil population, females suffering from surface cancer have a higher ratio of melanoma than males; both women and men have a lower ratio of melanomas than observed among soldiers and sailors.

[1] Having followed up 1,149 patients with skin cancer for 5 years, Warren and Gates (18) found noncutaneous cancers 4.5 times more frequently than they expected. The expectancy was strongly underestimated because a crude and incomplete cancer rate was used instead of an age-adjusted and complete rate, because the factor of cancer disposition was neglected, and because a key which may not be adequate was used for transformation of mortality into incidence rates. Moreover, they also counted as actual cases some in which the noncutaneous cancer could not have been prevented by the cancer of the skin because it occurred prior to the skin cancer.

3. On the average, patients with melanoma are younger than other patients with cancer of the skin or cancer in general. The ratio of melanoma to all surface malignancies drops with advancing age. The age specific incidence rates of melanoma rise more slowly than those of the other malignancies.

4. With regard to the incidence of melanoma the geographical or climatic factor plays a role different from that in the incidence of surface epitheliomas and of internal cancers.

5. The distribution of melanoma on the surface of the body differs materially from that of epithelioma. On the head, of all surface malignancies, including lips, 0.72 per cent were melanomas. Excluding the lower lip, the ratio rises from 0.72 to 1.0 per cent. On the other parts of the skin the corresponding value is 16.2 per cent, and in sailors and soldiers 34.6 per cent.

6. These facts indicate that for the change of a nevus into a melanoma, and for the higher incidence of melanoma in the armed forces other factors are responsible than those related to the pathogenesis of epithelioma of the skin and lip. With regard to melanoma the ultraviolet portion of the spectrum does not play a major role.

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The Antigenic Nature of Purified Chicken Tumor Agent*

Preliminary Report

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(Received for publication June 8, 1941)

The antigenic nature of the filterable causative agents of the fowl sarcomas have been the subject of several investigations during the past few years. Considerable confusion still exists, apparently because of three factors; namely, the inherent lability of the material, variations in the types of materials used, and comparison of preparations of widely variable degrees of purity. Though the early workers used relatively crude preparations of the agent, the complex antigenic nature of the material was already recognized. A summary of this early work was published by Murphy and his co-workers in 1932 (7).

The recently developed method of securing the tumor agent in relatively pure form has opened up the possibility of extending the investigation on the antigenic properties. Amies and Carr (1) used such purified products in the preparation of potent antitumor serums in rabbits but when they absorbed these serums with chick embryo pulp, the tumor-neutralizing properties were lost. Gye and Purdy (5) had previously reported contrary results when using antiserum derived from goats. The interpretation of these results is obscured by the fact that in the latter case the antitumor serum was prepared by the injection of tumor filtrates and in both cases crude preparations of chick embryo were used for absorption.

Claude (2) has succeeded in isolating the agent from chicken tumor I in what appears to be a high degree of purity with the tumor-producing activity unimpaired. The same method applied to chick embryo tissue (3) yields a fraction of similar composition made up of particles of the same size but without tumor-producing activity. With these two materials for antigens there is an opportunity for a more careful immunological study. The following experiments provide a partial answer to some of the questions concerning the antigenic properties of the agent of chicken tumor I.

* This investigation was carried on with the cooperation of Dr. James B. Murphy.

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Materials and Methods

The active particulate fraction was isolated from chicken tumor I by the method of differential sedimentation as developed by Claude (2). From each preparation samples were tested on chickens and proved to have a high tumor-producing activity. A similar particulate fraction was secured by the same method from 8-day-old chick embryos. These sediments were resuspended in phosphate buffer and injected intravenously into rabbits. The amount given was 0.2 mgm. of the sediment per kg. of body weight, a dose repeated at 48-hour intervals until 3 injections had been given. Three rabbits were used for each of the test materials. The one from each group showing the highest titer 8 days after the third injection was selected for a second course of 3 injections with a 50 per cent increase in dosage. After another 8-day period the rabbits were bled and the serums collected. The same procedure was repeated on a second group of 6 rabbits so that 2 antiserums for each of the antigens were available for the final tests.

The precipitin content of absorbed and unabsorbed serums was tested in homologous and heterologous systems. It was necessary to incubate these for 2 hours at room temperature and leave them overnight in the icebox. This may have been due to the extremely small quantities of materials involved. Suitable controls were run concurrently on all reactions.

Absorption of serums was carried out by mixing undiluted antigen and serum, allowing an incubation period, and then removing the antigen by high-speed centrifugation. Various dilutions and incubation periods were tried and it is believed that complete exhaustion was attained in the serums used for comparison.

The neutralizing properties of the serums were tested against potent tumor agent by intradermal inoculation in fowls. This was done by mixing serum in equal proportions with dilutions of the tumor agent and allowing the mixture to stand at room temperature for one hour. No complement was added. Each young

adult Plymouth Rock chicken used in this series received 8 intradermal injections of 0.4 cc. of this mixture. On one side four dilutions (10^0, 10^{-1}, 10^{-2}, 10^{-3}) of the tumor extract were used with a single serum. On the other side of the bird similar dilutions were used with a second serum with which the first was to be compared.

In evaluating the effects, the sizes of the resulting tumors were observed to be consistent with the dilutions used but judgment as to the neutralizing properties of the serums was based upon a comparison of the greatest dilution of the agent which produced a tumor on one side of a fowl with the greatest effective dilution on the other side of the same fowl. All comparisons were carried out in more than one fowl; two groups totaling 18 birds in all were used.

Results

Precipitins could be detected in the serums in high titer but the long incubation period already noted was necessary. A detailed report of the results of these tests would be without point since the differences observed were not great enough to form a trustworthy basis for comparison. It is true that there were equivocal results which suggested that, when antitumor serum was absorbed with embryo extract, something was left in the serum which could precipitate tumor extract but not embryo extract. On the whole, however, we considered that, in our hands, the precipitin reaction had not afforded an adequate basis for differentiating homologous and heterologous systems of these materials. This is in agreement with the findings of Kabat and Furth (6).

The presence of the Forssman antibody in rabbit serums, has been previously reported by Furth and Kabat (4). This antibody was present also in our serums. Absorption of the serums with washed sheep cells removed the hemolysins for sheep cells and the cross reactions between the two primary antigens disappeared but the antibody present in antitumor serum, which can be detected only by use of the homologous antigen, remained.

The results of the neutralization tests *in vivo* can be briefly stated as follows:

1. Antitumor serum neutralized tumor agent but normal rabbit serum did not.

2. Antiembryo serum showed no difference from normal serum; *i.e.,* neither neutralized the agent.

3. Antitumor serum neutralized tumor agent but antiembryo serum did not.

4. Antitumor serum neutralized tumor agent but antitumor serum absorbed with tumor extract did not.

5. Antitumor serum retained its neutralizing properties when absorbed with embryo extract but not when absorbed with tumor extract.

Discussion

The important findings here are (a) that antigenic differences between the purified extracts of chick embryo and chicken tumor can be observed; (b) that antiserum prepared by the use of purified embryo extract does not neutralize tumor agent *in vivo;* and (c) that the fowl specific and tumor specific properties of tumor antiserum can be dissociated under the above conditions.

Discrepancies between these results and those of others are probably due to the use here of highly purified products throughout. Furthermore, the antiserums used in these experiments were prepared by the use of minimal amounts of the antigen and could therefore be expected to exhibit a high degree of specificity with minimal cross reactions (8). The workers cited above (1, 5) used large amounts of antigen in a long course of immunizing injections.

Interpretation of these results at the present time would be premature. It may be that the immunologic difference observed represents an active substance in chicken tumor extract which is associated with, attached to, or adsorbed upon the particulate matter, or it may mean that the activity of tumor extracts resides in an altered normal cell constituent.

Conclusions

These experiments indicate that, when products of the highest degree of purity presently obtainable are used, there is a demonstrable immunologic difference between the extracts of chicken tumor and similar material from normal tissues.

Acknowledgement: Thanks are due to Dr. Karl Landsteiner and Dr. M. W. Chase for helpful advice and criticism.

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Growth of Brown-Pearce Carcinoma in the Anterior Chamber of the Eyes of Tumor-Immune Rabbits*

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The anterior chamber of the eye has long been recognized as a valuable site for the study of the growth of transplanted tumors. Recently Greene (8) successfully grew human cancers in the eyes of lower animals despite the fact that all other tissues were resistant to such heterologous tumor transplantation. The explanation for this may lie in the fact that a "barrier" exists between the blood and aqueous humor (7) which prevents the passage of antibodies from the former to the latter. This assumption is in some respects supported by the work of Becht and Greer (2) and also that of Hektoen and Carlson (9) who studied the concentration of antibodies in the various body fluids of animals immunized to bacterial and other foreign proteins and found that the titer of these immune bodies was highest in the serum and that they were present in very low concentration or were absent from the aqueous fluid of the anterior chamber of the eye.

These facts suggested that tumor might be grown in the anterior chamber of the eyes of animals immunized to a particular tumor by intracutaneous transplantation with the same tumor (4, 5, 12), despite the fact that all other tissues of the host are resistant to the growth of this tumor. Besredka and Bardach (3) were unsuccessful in their attempts to grow Brown-Pearce carcinoma in the anterior chamber of the eyes of tumor-immune rabbits, although the same tumor grew readily in this location in normal rabbits. Nevertheless, in view of the other evidence mentioned above (2, 8, 9), it was thought advisable to make a detailed study of the fate of transplants of Brown-Pearce carcinoma in the anterior chamber of the eyes of rabbits immunized to this tumor.

Experimental Procedure

Thirteen mongrel brown-grey rabbits were used for this experiment. Transplantation into the skin covering the dorsal spine was carried out by the technic previously described (1). The tumors grew successfully in the skin of all animals receiving tumor transplants. They attained their maximum size varying from 2.2 × 1.8 cm. to 6.1 × 5.4 cm. within from 3 to 5 weeks and then began to regress. In all 13 rabbits the skin tumors were completely resorbed within 3 weeks after they had started to regress. Following the disappearance of the skin tumors, the Brown-Pearce carcinoma was reimplanted into the testes of all of these animals in order to verify their resistance to the growth of this tumor. None of these 13 rabbits showed any evidence of tumor growth following this second transplantation. For purposes of control, portions of the same tumor which was used for this second transplantation were also inoculated into the testes of 3 normal rabbits. All 3 of these control animals developed large testicular tumors.

The tissue used for intraocular transplantation was obtained from a metastatic nodule of the Brown-Pearce carcinoma in the liver of a rabbit. The tumor was removed aseptically, immersed in Tyrode's solution, and cut into fragments 0.5 to 1.0 cm. in diameter. The fragments used for transplantation were all obtained from the same metastatic nodule.

The eye was grasped with a forceps, rotated downwards so that the corneo-limbal junction was readily available, and an incision was made through the cornea at the limbus into the anterior chamber with a sharp keratome. After a small amount of aqueous fluid had escaped, a tumor fragment, grasped in a small curved forceps, was introduced into the anterior chamber. For purposes of control, portions of the same tumor tissue were transplanted into the anterior chambers of the eyes of 4 normal rabbits using identical technic to that described above.

Results

In the 4 normal animals used for controls the tumor fragments transplanted into the anterior chambers of the eyes began to grow within 7 days of the time of transplantation and within 3 weeks had completely filled the eyes.

* This investigation was aided by grants from the Women's Board of Michael Reese Hospital and the Ivan and Hannah Grunsfeld Fund.

The behavior of the tumor transplants in the tumor-immune animals was different. In 3 of the 13 rabbits the transplanted fragments became pale, the edges rounded, and the fragments gradually diminished in size and were resorbed within 3 weeks. Histologic examination of these eyes failed to reveal any tumor at the site of transplantation. In 10 of the 13 rabbits the transplanted fragments became vascularized within a period varying from 6 to 14 days. No increase in size of the transplants was observed, however, for at least 3 to 6 weeks following transplantation. In 2 of these 10 animals this latent period was prolonged to 8 weeks. Once instituted, growth of the transplants progressed at a much slower rate than in the control animals. Ultimately however, within a period varying from 2 to 4 weeks after the tumors started to grow, the eyes were filled with a mass of tumor similar in every respect to that in the anterior chambers of the eyes of the control rabbits.

Discussion

Davson and Quilliam (7) have stated that "there is a great deal of evidence that the aqueous humor is a filtrate from the blood plasma, molecules of the size of serum albumen or larger being retained while crystalloids are distributed between the two fluids in a manner characteristic of ultra-filtrates in vivo." These authors referred to a "barrier" between the blood and the aqueous humor. It is conceivable that this "barrier" is capable of preventing the passage of antibodies from the blood plasma to the aqueous fluid thus allowing tumor transplants to grow in this fluid when all other tissues are resistant to their growth. This view is supported by the experiments of Becht and Greer (2) and Hektoen and Carlson (9), mentioned above.

It has frequently been stated that humoral antibodies have never been demonstrated in rabbits immune to Brown-Pearce carcinoma (6). However according to Lumsden (11) regression of tumors in rats and subsequent immunity was associated with a rise in the titer of agglutinins in the serum for this particular tumor protein. Kidd (10) has described a complement-fixing antibody in the blood of rabbits bearing the Brown-Pearce carcinoma and also in rabbits in which the growth had regressed. It is not unlikely therefore that other immune bodies for the Brown-Pearce carcinoma appear in the blood of immunized animals even though serologic tests employed at the present time are unable conclusively to demonstrate them. It is also possible that, associated with this rise in the titer of immune bodies for this particular tumor protein in the blood, they also appear in markedly reduced concentration in the aqueous fluid. While the titer of these immune bodies in the aqueous humor of tumor-immune animals may be sufficient to retard the growth of the tumor transplants in the anterior chamber of the eye, they are apparently not present in sufficient concentration to suppress completely the growth of these transplants as occurs in all other tissues of the body in such refractory animals. In this connection it should be mentioned that in 3 of our 13 rabbits the tumor transplants did not grow at all in the aqueous fluid of the anterior chamber. This is probably due to the fact that sufficient antibodies were able to pass from the plasma to the aqueous fluid to suppress the growth of the tumor in the anterior chambers of the eyes in these animals.

In a previously well controlled study (13) it was shown that not only can rabbits be rendered refractory to the growth of the Brown-Pearce carcinoma by regression of intracutaneous tumors, but also that intracutaneous growth and regression of this tumor causes disappearance of pre- and co-existing testicular tumors and their metastases. In these studies we observed that occasional metastases within the eye also regressed following regression of the skin tumor. If it is true that the aqueous humor of the anterior chamber of the eye in the tumor-immune animal contains no antibodies for the Brown-Pearce carcinoma, or a very low concentration of them, the regression of pre-existing eye tumors in the refractory animal is difficult to explain. One would expect that following active immunization against this tumor, regression of the carcinoma would occur in every tissue except in the anterior chamber of the eye because of the supposed lack of immune bodies in this location. It is of course possible that the regression of pre-existing eye metastases in the immunized animal may have been a spontaneous coincidental regression in no way whatsoever related to, or dependent upon, the regression of the skin tumor. However we must emphasize that in our experience with the Brown-Pearce carcinoma over a period of 6 years, spontaneous regression was only rarely encountered.

The failure of Besredka and Bardach (3) to obtain successful intraocular growth in previously immunized animals may have been due to the fact that insufficient time was allowed to elapse from the time of transplantation to the termination of the experiment. As pointed out above, the latent period from the time of transplantation into the anterior chamber until the appearance of growth is markedly prolonged in the immune animals. In 2 animals, 8 weeks elapsed before the transplanted fragment showed any increase in size in contrast to the control animals where growth began usually after a latent period of 4 to 7 days.

Summary and Conclusions

Immunization of rabbits to the Brown-Pearce carcinoma by intracutaneous transplantation does not

confer protection against the growth of this tumor in the anterior chamber of the eye, despite the fact that all other tissues tested are resistant to its growth following such intracutaneous transplantation. However the growth of the tumor transplants in the eyes of such tumor-immune rabbits is considerably delayed.

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Cellular Injury in Relation to Proliferative and Neoplastic Response*†

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(Received for publication May 31, 1941)

The diversity of methods and of agents capable of inducing neoplastic responses in animals suggests that the final result is not a direct effect by the agent, but rather an effect on the cell which in turn may liberate as a result of injury or metabolic disturbance an active proliferative or growth-promoting factor. The present series of experiments has been directed toward determining the relationship of prolonged tissue injury to marked proliferative response leading perhaps ultimately to the development of experimental neoplasia.

The relation of chronic inflammation to the development of tumors is a clinical observation which has been described by numerous investigators. Ewing has made an excellent survey of the problem (8). Various forms of chronic inflammation, according to Ewing, have been observed to precede the appearance of numerous tumors. Precancerous conditions represent in many instances various forms of chronic inflammation. These may be manifested in several different forms such, for instance, as cicatrices from burns, syphilitic, or tuberculous lesions. Wolbach studied the effects of repeated roentgen ray exposures upon the skin antecedent to the formation of carcinoma (26). He pointed out that the reactions of the epidermis are probably secondary to lesions in the corium. Following x-ray injury, for many years the epidermis proliferates and behaves as in any ordinary reparative process. But continuous proliferative activity in an abnormal supporting tissue predisposes to the development of malignant properties (26). After reviewing many of the forms of precancerous inflammatory conditions Ewing (8, p. 527) arrives, for instance, at these conclusions: "In routine material I find transition stages from hypertrophic endometritis to carcinoma rather frequently, but clinical observations are still inadequate to prove the close dependence of the malignant upon the inflammatory process." In discussing physiological involutions of organs favoring neoplastic development Ewing (8, p. 529) states that "In the prostate the chief condition predisposing to cancer is chronic prostatitis usually with hypertrophy." In regard to the transformation of benign into malignant tumors, Ewing concludes that some benign growths appear to originate from inflammatory processes and that the involved tissues may present all gradations between simple proliferative activity, adenoma, and carcinoma. In discussing the factors which incite malignant growth of embryonal cells, Ewing (8, p. 530) concludes that "one may reasonably search for them among the causes of local hyperemia." In a recent review Laurens (17) has pointed out that since carcinogenesis by ultraviolet irradiation is usually preceded by severe erythema, it is possible that the production of neoplasia is related to chronic inflammation. The influence of an inflammatory reaction on the subsequent development of neoplasia has been a controversial subject. For instance, Deelman (6) has expressed the view that the latent period in the production of tar cancer in mice is shortened by repeated scarification of the tarred areas. On the other hand, Roussy and others (19, 21, 22) have failed to confirm any such hastening in development. Beck (1) has found that acute inflammation has apparently no influence on the rate of absorption and on the carcinogenicity of 3,4-benzpyrene in fatty solvents. He failed to obtain any sarcoma from the fibrous tissue resulting from subacute inflammation. Brunschwig and his associates (3) likewise have been unable to establish that injury to tissue in mice enhances their susceptibility to carcinogenic hydrocarbons. The studies of Lacassagne and later of Burrows have emphasized, however, the role of inflammation in influencing carcinogenesis. Lacassagne (15) infected rabbits with a microorganism, *Streptobacillus caviae*. The infection subsided under the influence of irradiation. Six months to 4 years later, tumors were found to have developed at the site of irradiation. From these observations Lacassagne concluded that local inflammation seems to be an important factor in carcinogenesis. Burrows and his collaborators (4) have essentially confirmed these observations. They have induced foci of inflammation in the groin of rabbits by injection of kaolin or silica suspended in olive oil. These areas were subsequently irradiated. Within 2 years tumors were found to have developed in 6 out of 9 animals. Suntzeff, Babcock, and Loeb (25) have observed the development of sarcoma in mice repeatedly injected subcutaneously with 0.5 per cent of buffered HCl solution for a period of 12 to 15 months. Similar injections with distilled water, Na_2SO_4, glycerine, or gelatine have failed to induce any such response.

The studies of several investigators support the view that either with or without concomitant neoplasia an active carcinogenic substance can be extracted from an otherwise uninvolved liver tissue. This possibility has been demonstrated by Hieger (12), Des Ligneris (7), Shabad and his co-workers (13), and more recently by Steiner (24) in patients with carcinoma.

The production of tumors in rabbits with carcinogenic hydrocarbons has not yielded uniform results in the hands of various investigators.

Some workers (14) have succeeded in obtaining squamous carcinoma in the ears of rabbits after painting with benzpyrene. Others (16) have failed to obtain any such results. The writer has observed that several rabbits have been unable to develop any neoplastic response following repeated painting of their

* This investigation was aided by grants from The International Cancer Research Foundation and from the Dazian Foundation for Medical Research.

† Read at the 34th Annual Meeting, American Association for Cancer Research, Inc., Chicago, Illinois, April 16, 1941. A brief abstract appears also in the Eighth Scientific Report of the International Cancer Research Foundation, p. 74. 1940.

ears with 0.3 per cent benzpyrene in benzene for periods ranging from about 8 to 11 months. Yet, benzpyrene is the active hydrocarbon recovered in coal tar which in turn, as is well known, induces the characteristic tar cancer of rabbits. This fact in itself suggests that the benzpyrene *per se* as found in crude tar may well have little to do with the carcinogenic action of tar repeatedly applied to the ears of rabbits. It is to be remembered in this connection, however, that Shear (23) and more recently Berenblum (2) have emphasized the presence of so-called cocarcinogens in a fraction of creosote oil or in croton resin which may activate carcinogens. Wolbach (27), studying on mice the response of carcinogenic chemicals antecedent to tumor formation, arrived at the conclusion that none of the chemicals employed (hydrocarbons and 2-amino-5-azotoluol) owed their carcinogenic property to direct stimulation of cell growth.

The observations of a number of investigators indicate that injured tissues liberate growth-promoting substances.

Carrel (5) studied this problem in relation to tissue culture and to wound healing. He pointed out the presence of such substances in embryonic tissue juices, in extracts of adult tissues, such as muscle, thyroid and kidney, in extracts of leukocytes, and apparently in nonsuppurative inflammatory exudates. He expressed the view that lymphocytes function as a storehouse of embryonic growth-promoting substances or trephones. Observations made by Fischer (10) support the view that injured cells liberate growth-promoting substances to a greater extent than do completely destroyed cells. Loofbourow and his collaborators (9) have likewise demonstrated that yeast cells injured by low intensity ultraviolet irradiation liberate a greater amount of proliferation-promoting substances than similar cells killed by exposure to higher intensity. These latter investigators expressed the opinion that such proliferation-promoting factors may afford a clue to the explanation of wound healing and even possibly to certain types of malignancy (9). Thus the proliferative-promoting substances may essentially be related to the substances or so-called "wound hormone" of Marchand (20), and of Haberlandt (11). Spectroscopic analyses have indicated that the factors have an absorption near 2,600 A. characteristic of purines and pyrimidines of the nucleic acid complex. The active factors are diffusible through a dialyzing membrane leaving an inactive residue behind.

The foregoing brief résumé of the pertinent literature indicates that cellular injury, as manifested, for example, by inflammation, is probably concerned with the ultimate proliferative or reparative phase. It is conceivable that this reaction is largely referable to the liberation of one or several factors from damaged cells. That an exaggerated proliferative response over a protracted period may influence ordinary repair and thus favor neoplastic development is a distinct possibility. Certainly the diversity of means employed to induce formation of tumors renders worthy of consideration the concept of a common denominator liberated by cells whose physiological equilibria and organization have been profoundly disturbed, either through long-standing injury or else through metabolic alteration. The series of observations presently to be described is a first attempt in this direction. With further progress along this line, additional reports will follow.

Experimental

An inflammatory exudate may be considered as a fluid containing products of cellular injury, besides some degree of admixture from elements of the circulating blood. Consequently the exposure for a long interval of normal cells to such material may conceivably influence their subsequent fate.

The technical procedure adopted follows briefly: Rabbits were repeatedly injected subcutaneously once or twice a week at the base of one ear with 0.5 cc. of exudative material. The exudate was obtained from rabbits with an acute inflammation induced in the pleural cavity by the introduction of about 0.2 cc. of 5 per cent croton oil in olive oil. The period of injections ranged from 4 to 7½ months with an occasional interruption lasting from about 1 to 3 weeks. At first an acute inflammatory reaction developed at the site of injections. The suppurative material in the abscess was found, upon culturing on broth, to be sterile.

Following the termination of these repeated injections of exudate the local suppurative reaction eventually subsided and was gradually succeeded by firm, indurated, small and irregularly shaped palpable masses at the site of injections. These tiny nodular prominences failed to regress 6 to 8 months after cessation of injections (Fig. 1). There was even a suggestion of very slow but nevertheless distinct increase in size.

Biopsies of the areas in question were made for microscopic examination. For anatomical orientation, a cross section of the site of study in the normal ear of a rabbit is shown in Fig. 2. The epithelial layer and the cartilage plate show no abnormality. In contrast, biopsies of nodular prominences or of the firm cartilaginous masses in ears of experimental animals, months after cessation of injections of exudate, reveal marked proliferative activity on the part of the cartilage. Newly formed cartilage was found deposited in abundance and in an irregularly-shaped pattern branching off from the main cartilage plate (Fig. 3). In addition, cystic formation and in some cases areas of metaplastic ossification with islets of hematopoietic activity were also noted (Figs. 4 and 5).

Morphological criteria seem insufficient for establishing with certainty what might be termed autonomous growth. Therefore it is difficult to assert whether the marked proliferative response on the part of the cartilage described above had reached this stage (Fig. 6). The probability is that, at least in the foregoing preparations, this had not yet been attained. Other biological tests, such, for instance, as transplantation will finally aid in determining this point. It is to be recalled in this connection that over 7 months following cessation of the course of injections of the exudative material, at a time when there was no trace

of a remaining abscess, the cartilaginous protuberances had failed to regress (Fig. 1). Nevertheless in spite of the marked and sustained proliferative response, it is as yet difficult to diagnose the lesion with certainty as a true chondromatous structure. There are, however, exceptions to this conclusion, at least in the present series of animals. For instance, in the ear of an experimental rabbit a month following a biopsy adjacent to the site of repeated exudate injections, a tiny papillomatous lesion and a small nodular protuberance were found to have developed. Histological examinations of the latter revealed a structure whose cartilaginous pattern was wholly different from that of the original plate and at the same time perfectly consistent with a chondroma (Fig. 7). These studies are being pursued and extended further.

The data bearing on the observations are conveniently assembled in Table I. It is to be noted that in most of the animals, the areas of the ear repeatedly injected with exudative material revealed not only marked irregular cartilaginous proliferation, but also atypical epithelial hyperplasia with frequent foci of hyperkeratosis. This is particularly well illustrated in Figs. 8 and 9. The epithelium tends to dip down into the corium in the form of irregularly shaped, tongue-like projections in the center of which keratin formation appears as a characteristic part of the picture. This proliferative reaction is in sharp contrast to the normal epithelial layer found in the untreated ear of rabbits (Fig. 10). The pattern assumed by the epithelium of ears of rabbits receiving for several months repeated injections of exudate is doubtless of interest especially when one bears in mind various types of precancerous conditions. In this connection it is noteworthy that in the series of animals studied, a few rabbits were found to have developed small papillomatous-like tufts projecting outward for several millimeters. These were observed to have originated in the area of skin adjacent and in direct apposition to the original site of the abscess that had developed during the course of injections of exudate (Fig. 11). In a rabbit (No. 21-01) which, a month subsequent to biopsy of the exudate-treated area, had developed a chondroma (Fig. 7), a papilloma-like structure was found adjacent to the chondroma which on section revealed a pedicle surmounted by a growth lined with hyperplastic epithelium (Fig. 12). An additional unusual feature for a typical papilloma was the presence of sebaceous glands. These structures, however, were found frequently to be likewise considerably hyperplastic in the ears of animals repeatedly injected with exudate.

The foregoing observations indicate quite clearly that numerous injections in the rabbit ear of products of cellular injury as found in inflammatory exudates induce significant local changes in both cartilage and epithelium. It is conceivable that the marked proliferative response would yield even more striking results if the course of injections were lengthened. This phase of the work is now being investigated. A similar study is also being undertaken in strains of mice susceptible to tumor formation.

Two types of controls were utilized. To rule out the nonspecific effect of parenteral protein injections, rabbit serum was repeatedly injected for months into the subcutaneous tissue of the ear of rabbits. The results obtained are listed in Table I. It is clear that with serum injections none of the proliferative type of response elicited with exudates was ever encountered (Fig. 13). This would suggest that the factor responsible for the marked proliferative effect probably originates from injured cells. There is, however, a possible objection to such an inference. Serum injections fail to induce a local inflammatory reaction. This fact in itself may be responsible for the absence of subsequent marked reparative response. An attempt was therefore made to obtain further information on this point. In other words, is the marked proliferative effect discerned months after cessation of injections of exudate referable to previous direct injury of cartilage and epithelium or is it due to a protracted and cumulative exposure of these tissues to some proliferative factor present in small amounts in exudates?

A few rabbits were repeatedly injected in the subcutaneous tissue of the ear for about 4 months with

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Photograph of ear of rabbit No. 20-93 showing the nodular prominences which developed approximately 6½ months after the last injection of exudate. During the preceding period of 7½ months, 38 injections of material from exudates were made into the subcutaneous tissue of the external ear.

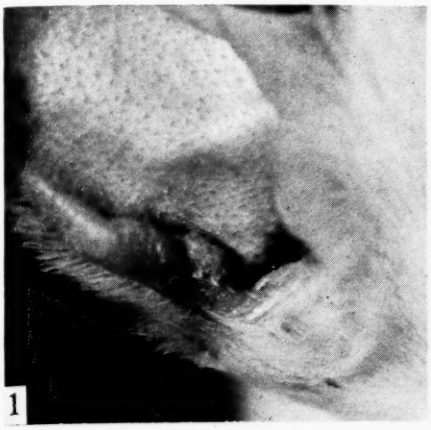
FIG. 2.—Drawing of microscopic section through the normal ear of a noninjected rabbit showing the typical epithelial covering and cartilage plate. Mag. × 80.

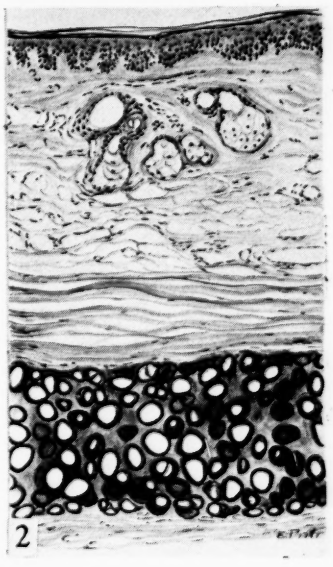
FIG. 3.—Photomicrograph of section of the ear of rabbit No. 21-06. This animal received 32 injections of exudate into the subcutaneous tissue of the ear during a period of 6½ months. Approximately 7 months after cessation of injections palpable firm masses appeared. These were produced by marked proliferation of the cartilage forming the irregular branching pattern shown in this photograph. Mag. × 85.

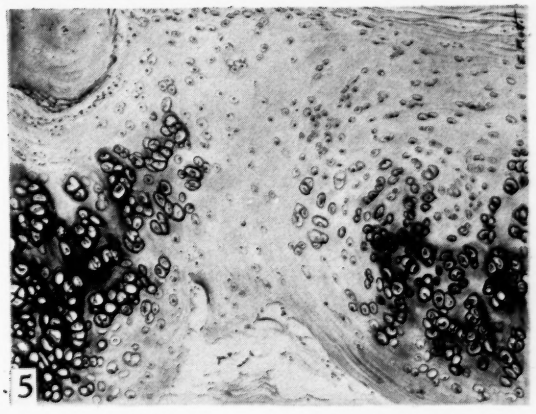
FIG. 4.—Photomicrograph of section of nodule in ear of rabbit No. 20-26, 6 months after cessation of exudate injections, showing an area of metaplastic ossification with islets of hematopoietic activity. Mag. × 127.

FIG. 5.—Drawing of section of ear of rabbit No. 20-26 showing marked proliferation of cartilage with an area of ossification. Mag. × 60.

FIG. 6.—Photomicrograph of section of the ear of rabbit No. 21-01 showing marked proliferative changes in the perichondrial region 7½ months after cessation of a series of 20 injections of exudate during 4 months. Mag. × 85.







FIGS. 1-6

TABLE I: EFFECT OF REPEATED INJECTIONS OF EXUDATES ON CARTILAGINOUS AND EPITHELIAL PROLIFERATION IN THE EARS OF RABBITS

Animals injected with exudate						Animals injected with either serum or an emulsion of serum with croton and olive oil					
Rabbit No.	Approximate period of injection, in months	Total number of injections	Approximate interval between last injection and excision of site of injections	Proliferation of cartilage	Hyperplasia of epithelium; hyperkeratosis	Rabbit No.	Approximate period of injection, in months	Total number of injections	Approximate interval between last injection and excision of site of injections	Proliferation of cartilage	Hyperplasia of epithelium; hyperkeratosis
20-28	1	7	1 day	+	++	20-92	6½	30	14 days	0	0
20-59	4	18	5 days	0	+	20-85	7½	38	6½ months	0	0
20-63	4	19	14 days	Trace	0	21-24	6½	32	7½ months	0	0
20-77	7½	38	24 days		++	20-45	7½	38	7½ months	0	0
20-26	6½	32	6 months	+++ Also metaplastic ossification	+	21-02	6½	32	7½ months	0	0
20-93	7½	38	6½ months	++	+	20-71	4	20	7½ months	0	0
20-57	4	20	6½ months	+ (in the gross)	Papillomatous tuft	21-00	6½	32	7½ months	0	0
21-06	6½	32	7 months	+++	++	21-42	4	20	8½ months	0	0
21-17	7½	38	7 months	+	0	21-08	7½	38	8½ months	0	Trace
21-09	7½	38	7½ months	Trace	+ to ++	21-11*	4	15*	7½ months	0	0
21-01	4	20	7½ months	++	+	21-13*	4	15*	7½ months	0	0
						21-23*	4	15*	8 months	Trace	

* Animals repeatedly injected with serum emulsified with croton and olive oil.

the irritant in the form of croton oil and olive oil, emulsified with rabbit serum. This was prepared by shaking 5 cc. of serum with 0.5 cc. of 5 per cent croton oil in olive oil. Of this material, 0.5 cc. was injected repeatedly once to twice a week into the subcutis of the ear precisely as in the case of exudate or serum. This type of treatment induced a very severe local inflammatory reaction leading to abscess formation. After cessation of the course of injections, the acute reaction gradually subsided. Several months later biopsy of the now apparently normal site failed to reveal any striking abnormal proliferative response on the part of either cartilage or epithelium (Table I, Fig. 14). In the ear of only one rabbit a mild degree of cartilaginous proliferation was found in the perichondrial region. Two factors may possibly explain

DESCRIPTION OF FIGURES 7 TO 11

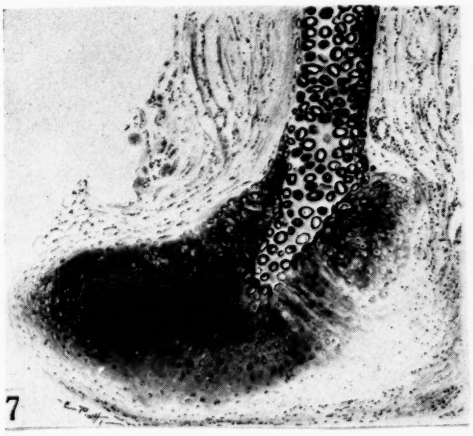
FIG. 7.—Photomicrograph of section of the ear of rabbit No. 21-01 showing probable chondroma. This rabbit received 20 injections of exudate during 4 months. Tissue removed at biopsy 7½ months later showed structures shown in Fig. 6. One month after excision of the tissue and in an adjacent area a papillomatous structure and chondroma developed. Mag. × 60.

FIG. 8.—Drawing of microscopic section through the ear of rabbit No. 20-28 at the site of 7 successive injections of exudate. Note the hyperplasia of the epithelium dipping downward into the corium and the foci of keratin formation. Mag. × 80.

FIG. 9.—Photomicrograph of section of the ear of rabbit No. 21-06 showing besides proliferation of cartilage (see Fig. 3) and hyperplasia and keratinization of the epithelium. Mag. × 127.

FIG. 10.—Photomicrograph of normal ear of rabbit No. 21-06 for comparison with Fig. 9. Mag. × 127.

FIG. 11.—Photograph of ear of rabbit No. 20-57, 6½ months after the end of a series of 20 injections of exudate given during 4 months. Small papillomatous projections were present in the region of the ear constantly abutting against the site of acute suppuration that prevailed during the period of exudate administration.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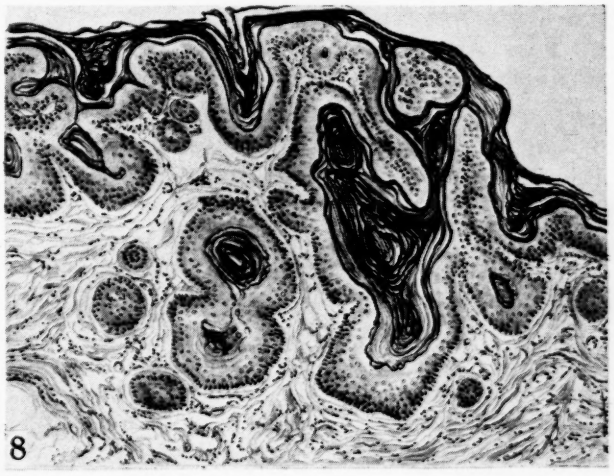
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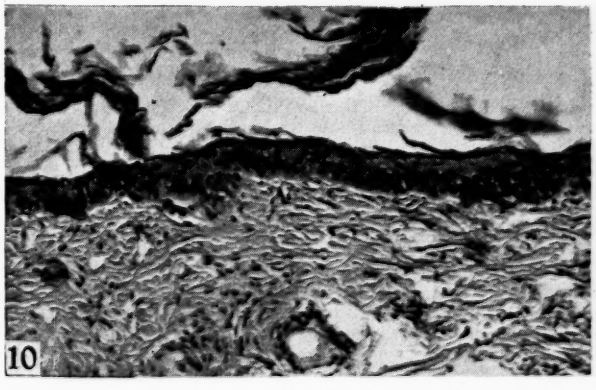
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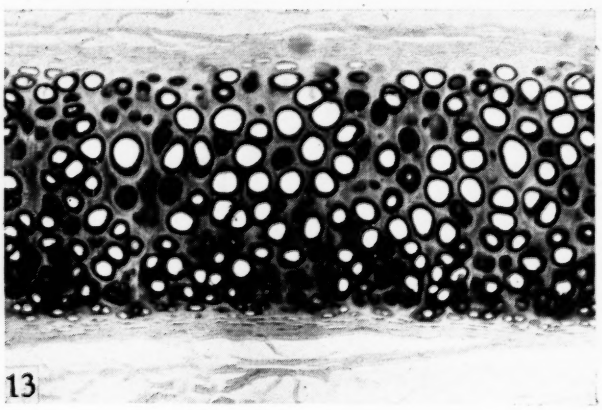
FIGS. 7-11



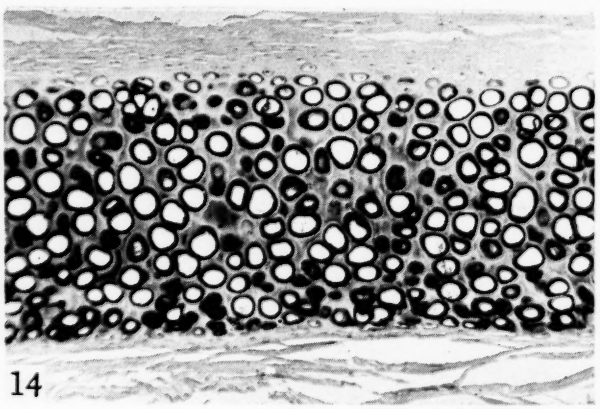


FIG. 12.—Photomicrograph of section of papilloma of the ear of rabbit No. 21-01 showing hyperplasia of the epithelium and inclusion of sebaceous glands in the papilloma. The animal had received 20 injections of exudate adjacent to the site of this neoplastic structure during 4 months. Proliferation of cartilage and hyperplasia of epithelium were conspicuous $7\frac{1}{2}$ months after cessation of injections. A biopsy was performed. One month later, adjacent to the site of excision, a chondroma (Fig. 7) and this papilloma were found. Mag. × 127.

FIG. 13.—Photomicrograph of section through the cartilage of the ear of rabbit No. 21-01, $7\frac{1}{2}$ months after cessation of a course of 32 injections of serum during a period of $6\frac{1}{2}$ months. Note contrast to the effects of the exudate injections, the complete absence of proliferative response. Mag. × 85.

FIG. 14.—Photomicrograph of section through the cartilage of the ear of rabbit No. 21-13, $7\frac{1}{2}$ months after cessation of a course of 15 injections of croton oil in olive oil emulsified in serum and administered during a period of 4 months. Severe suppurative inflammation had occurred and receded. Note absence of any marked proliferative reaction. Mag. × 85.

this failure of marked proliferative reactivity to occur on the part of tissues subjected for several months to repeated injections of the irritant *per se*. In the first place, the intensity of induced local injury resulting from the direct and repeated destructive effect of the severe irritant would tend to yield a relatively negligible amount of reparative factor that would be at all effective. It is of interest in this connection to recall the observations of Fischer cited above (18) which indicate that injured cells liberate growth-stimulators to a greater extent than completely destroyed cells. In the second place, the relative concentration of the proliferative factor both in the exudate and in the emulsion of irritant and serum must doubtless be of an entirely different order. The exudate presumably already contains some of the growth-promoting factor, whereas it is either absent or at least occurs in minimal amounts in the serum and irritant emulsion. Thus, the capacity to induce intense injury by the irritant and, on the other hand, the higher concentration of reparative factor already present in the exudative material might well account for the striking differences obtained. The inability of the irritant to induce sustained and marked proliferative response in spite of pronounced local injury, therefore, definitely suggests that the effects obtained by successive injections of the exudate cannot be readily explained as an after effect due to primary injury of the perichondrium or epithelium by the acute inflammatory reaction *per se*.

These studies are being continued in an endeavor to elucidate further and with greater precision from a chemical standpoint the relation of products liberated by injured cells to both proliferative or reparative and perhaps neoplastic types of tissue response.

Conclusions

An inflammatory exudate is considered as a fluid containing products of cellular injury.

Frequent injections of an exudate into the subcutis of a rabbit's ear over a protracted period of time induce a severe inflammatory reaction. Cessation of these injections is followed months later by sustained and marked proliferative activity. This is characterized by atypical epithelial changes as evidenced by hyperplasia and metaplasia of the normal epithelial layer. Foci of keratinization also form a conspicuous feature of the reaction. Furthermore, the cartilage of the ear, at the site of injections, manifests marked proliferative activity giving rise to small palpable, and indurated structures. Microscopically the cartilage frequently appears as irregular unevenly shaped cords of proliferative cells derived from the perichondrium. Both fibrous and hyaline cartilaginous cells form the essential elements of the proliferative reaction. Areas of metaplastic ossification with hematopoietic centers are, however, also in evidence in such areas. In several instances the marked proliferative activity on the part of the epithelium and cartilage has been found to be associated with papillomatous tufts and with morphological structures consistent with chondroma (Fig. 7).

Serum injections or the repeated administration of croton oil in olive oil emulsified with serum have failed to induce, following acute inflammation, any marked proliferative responses.

The available evidences suggest the presence of one or possibly several proliferation-promoting factors liberated by injured cells into inflammatory exudates to explain the foregoing observations. These studies are being continued further in an endeavor to elucidate the nature of such growth-stimulating substances.

Technical assistance given by Mr. Sheldon C. Sommers is acknowledged by the author.

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The Resistance of Tumor Cells in Tissue Culture to the Growth-Inhibiting Action of Amines

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(Received for publication May 29, 1941)

During an investigation of those fractions of liver which are responsible for its growth-inhibiting action *in vitro,* the observation was made (4) that ethanolamine (colamine) has marked inhibitory activity. This compound was isolated from one of the most active fractions obtained. The present communication is a report on experiments made to define the mode of action of ethanolamine upon growing tissue, and to determine what related compounds show similar activity. It has been found in general that simple aliphatic amines are active, but that the activity is lost whenever a ring structure is present in the molecule.

Primary amines are compounds with the generalized formula RNH_2 and ammonia may be considered the simplest such compound. The NH_2 group confers basic properties, and those amines which are basic are neutralized by acids with addition of a hydrogen ion to the amino group. The ratio of activities $\frac{RNH_2}{RNH_3^+}$ may be considered unity at a point corresponding to the dissociation constant of the amine (K_B) where this is equal to the hydroxyl ion concentration of the medium. As the medium becomes more or less alkaline, the proportions of the two forms change in accordance with the mass law formula

$$\frac{(RNH_3^+)\times(OH^-)}{(RNH_2)}=K_B.$$

Secondary and tertiary amines have the same properties, but one or both of the hydrogen atoms of the NH_2 group are replaced by radicals.

The simpler amines usually have a pressor action, as shown by Barger and Dale (2); this action culminates in adrenalin and its homologues. Some of the amines; *e.g.* choline and ethanolamine, may lower blood pressure (8).

The part played by the amines (excepting ammonia and the amino acids) in tissue metabolism is little known. Ethanolamine and choline are present in the phospholipids, kephalin and lecithin, being linked to the remainder of the molecules through a phosphoric acid radical. The phosphoric ester of ethanolamine has been isolated from tumors (14) and from normal intestinal mucosa (6). It has been shown (17) that free choline probably does not exist in any appreciable quantity in liver tissue freshly removed from the body, but appears later as a post-mortem artifact. The same may be true of ethanolamine. Our meager knowledge of the amine content of tissue is in part due to the difficulty of estimating amines in the presence of ammonia or amino acids.

The action of amines on tissue cultures has been observed by Funayama (9) to consist in stimulation at low concentrations followed by inhibition at higher concentrations, a combination of effects which he has noted in many organic compounds. Vacuolation (thought to be fatty) and a striking cumulative action were noted. Amino acids have long been considered (1) inhibitory to growth. Vogelaar and Ehrlichman (18) have found that glycine is inhibitory chiefly when in the absence of serum; these writers suggest that serum and other colloids act to protect the cell surface from the action of absorbed amino acids.

The action of ammonia upon cells in culture has been examined by Rosenfeld (15), who showed that exposure to ammonia vapor causes reversible liquefactive changes in the cytoplasm of cells undergoing mitosis.

Experimental

Cultures of embryo, adult, and tumor tissues have been grown by the hanging-drop, Carrel flask, and roller bottle (16) technics. In hanging-drop and flask cultures 20 to 25 per cent chicken plasma has been used, while in roller bottle cultures a thin layer of plasma adherent to the cover slip has been allowed to clot before the addition of liquid medium. In routine determination of growth inhibition, Tyrode solution has been used as the supernatant medium in control cultures, while amines have been added in experimental cultures. In other experiments where prolonged survival or growth of cultures were desirable, synthetic media containing Witte's peptone were used. The pH of cultures was controlled by addition of CO_2 or by means of M/15 phosphate buffers, and phenol red was used in culture media so that the pH of each culture might be determined daily. Daily measurements of cultures were made and growth inhibition was estimated by the technic described previously (3).

The amines were obtained from the Eastman Kodak Company, except where otherwise noted. The free amines were neutralized with HCl before use, and used therefore in the form of hydrochlorides. The solutions were sterilized by boiling. Some of the most volatile amines were found to be sterile as obtained from the bottle. In all cases concentrated solutions of

amine hydrochlorides were diluted with nutrient media for use.

Results

The results of experiments with ethanolamine will first be cited, as these comprise the bulk of our experiments and are representative of the effects of amines generally.

Dependence upon pH.—An extreme variability in the apparent inhibiting concentration of ethanolamine in early experiments on normal cells proved to depend upon variation in pH of the medium. It was found possible to vary the pH of control cultures between 7.0 and 8.4 without changing appreciably the growth margin of the cultures. Changing the pH of cultures containing ethanolamine from 7.0 to 8.0 resulted in approximately a tenfold increase in the effectiveness of the amine, in causing partial or complete inhibition of growth. At pH 7.4, almost complete inhibition occurred with 0.6 mgm. of ethanolamine per cc. (10 millimoles per liter).

The correlation between dosage, pH of medium, and inhibition of growth is shown in Fig. 1.

In addition to neutralization of ethanolamine with hydrochloric acid, yielding the hydrochloride, neutralization was done with phosphoric, acetic, lactic, oleic, and stearic acids. The oleate and stearate were found to be extremely toxic to cultures, causing their destruction in concentrations below 0.05 mgm. per cc. This effect was duplicated by sodium oleate and stearate, and may reasonably be attributed to the high surface activity of soaps in general. The acetate, lactate, and phosphate produced the same effects as the hydrochloride per unit amount of ethanolamine. Neutralization with aspartic acid likewise caused no alteration in the action of ethanolamine.

Effects on morphology of cells.—The most striking influence of ethanolamine was an intense vacuolation of normal fibroblasts and epithelium alike (Figs. 2, 3, and 4). The vacuoles, which were not highly refractile, tended to fill the entire cell and to give it a honeycomb-like appearance. The nucleus, although not vacuolated, was frequently indented as if by pressure of surrounding vacuoles (Fig. 6). The vacuoles did not stain with hematoxylin or eosin, or with fat stains (Sudan III and Nile blue), neutral red, or Janus green. In Fig. 2 a fibroblast is shown which has been stained with neutral red, and the neutral red staining bodies can be seen in the reticulum between the vacuoles.

In order to gain further evidence of the aqueous nature of these vacuoles, cultures were made hypertonic and hypotonic by the addition of varying proportions of NaCl and water. The vacuoles were found to shrink or swell measurably under these conditions, as might be expected to occur if water were being exchanged to equalize osmotic pressure.

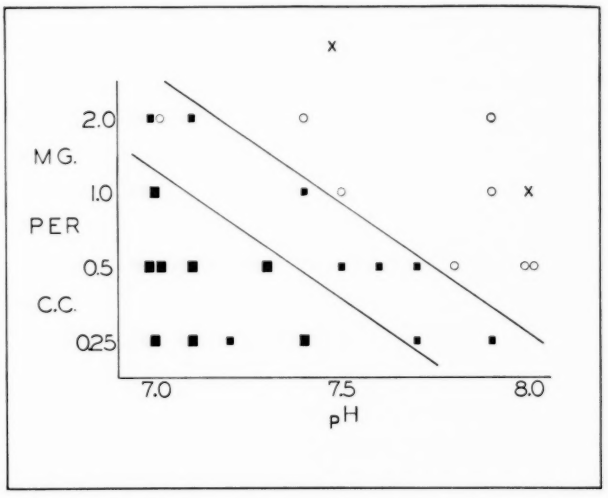


Fig. 1.—Variation in effect of ethanolamine with pH of culture medium and amine concentration. Large squares show normal growth of chick embryo cells. Small squares show vacuolation and retarded growth. Circles show no growth of embryonic cells. X, X equals approximate upper limit for growth of sarcoma 180 and mammary carcinoma of mouse.

Cultures of cells grown in Tyrode solution or growth-promoting (nutrient) media have been observed to take on the same vacuolar appearance when treated with ethanolamine solution or vapor. One mgm. of ethanolamine per cc. of medium at pH 7.4

DESCRIPTION OF FIGURES 2 TO 7 AND 9 TO 11

Fig. 2.—Two-day culture of chick embryo heart; treated by addition of ethanolamine 1:1,000 6 hours before, at pH 7.4. Vitally stained with neutral red. Mag. × 360.

Fig. 3.—Two-day culture of chick embryo liver, treated as in Fig. 2. Mag. × 70.

Fig. 4.—Three-day culture of chick embryo heart grown in triethanolamine 1:1,000, at pH 7.4. Mag. × 150.

Fig. 5.—Same culture as Fig. 4 2 days after washing off the amine solution, pH 7.4. Mag. × 150.

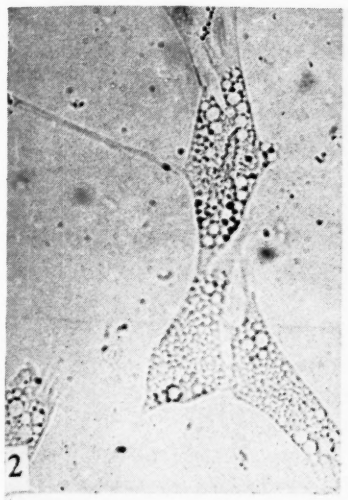
Fig. 6.—Three-day culture of chick embryo heart, grown in ethanolamine 1:2,000. Stained with Harris' hematoxylin. Note indentation of nuclei by vacuoles. Mag. × 500.

Fig. 7.—Nine-day culture of chick embryo heart in glycine 1:200. There is neither inhibition nor vacuolation. Mag. × 150.

Fig. 9.—Three-day culture of mouse subcutaneous tissue in ethanolamine 1:1,000, pH 7.3. There is marked inhibition of growth and slight vacuolation. Mag. × 150.

Fig. 10.—Control to culture shown in Fig. 9. Plasma and Tyrode medium alone, without ethanolamine. Mag. × 200.

Fig. 11.—Two-day culture of mouse sarcoma 180, in chick plasma and Tyrode solution. Control to 12. Mag. × 100.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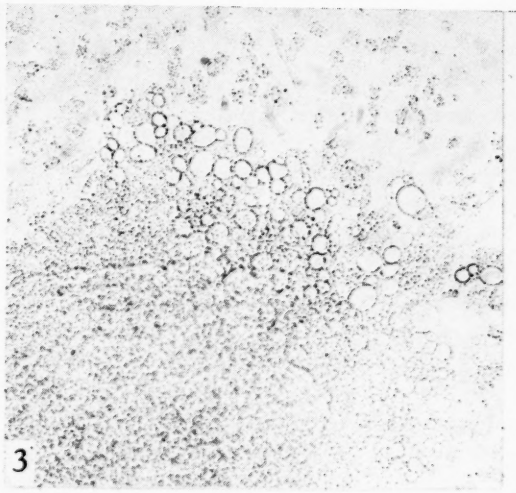
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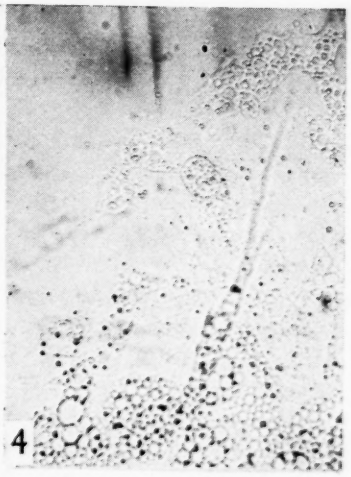
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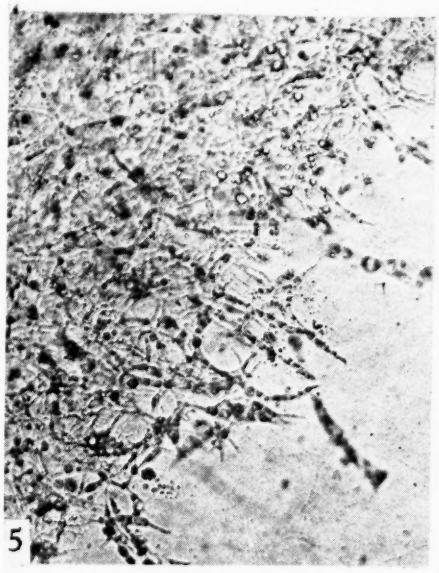
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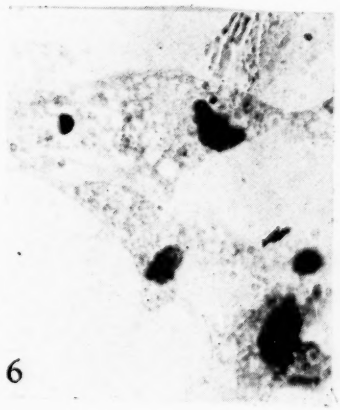
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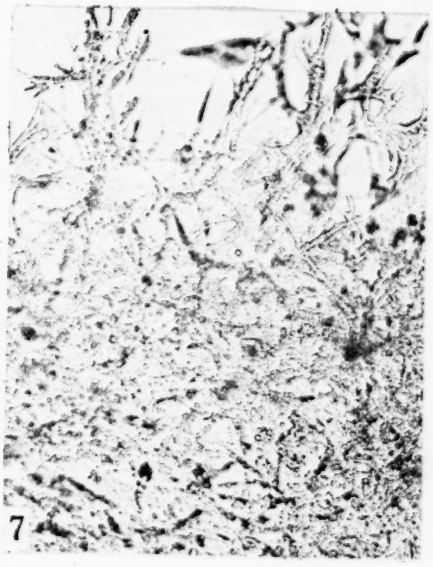
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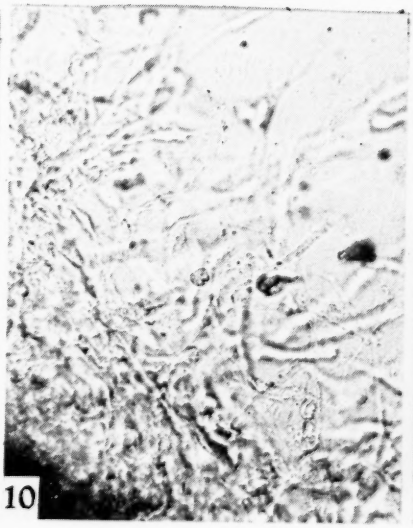
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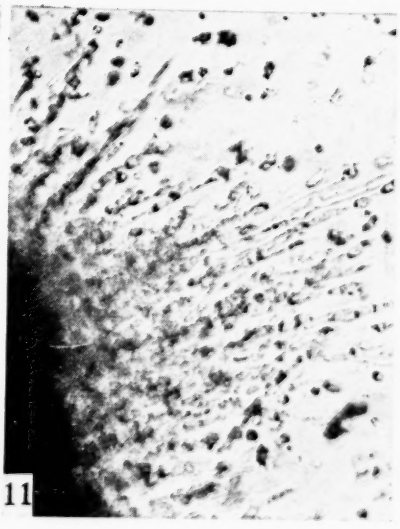
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FIGS. 2-7, 9-11

was seen to produce vacuoles within 2 to 4 hours. The vacuoles first appeared in scattered places within one hour and slowly increased in size without apparently coalescing. Higher concentrations of the amine (or treatment in a more alkaline medium) accelerated this process, and certain vacuoles reached a very large size. Those giant vacuoles, when located peripherally, often ruptured, and this was followed by cell disintegration.

Reversibility of the vacuolation was noted in cultures grown in, or treated by, ethanolamine. Reversal could be effected by washing off the solution or by making the medium relatively acid (Fig. 5). In either case, the vacuoles became smaller, and within a few hours disappeared. Reversibility of growth inhibition was also complete following removal of the amine (Figs. 4, 5, and 8).

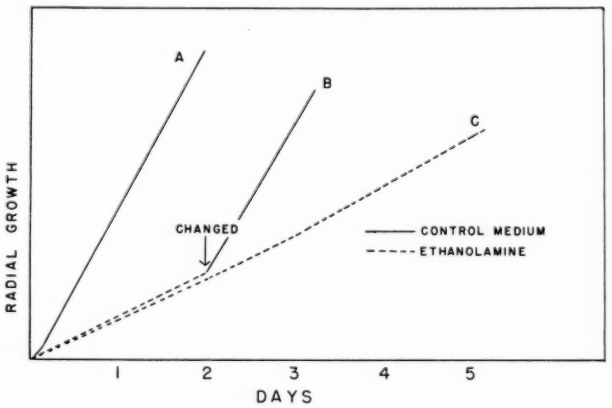

FIG. 8.—Radial growth of explants of chick embryo muscle in control medium (Witte's peptone and serum) and in the same medium with 0.5 mgm. ethanolamine added per cc. A shows culture in control medium. B shows culture in ethanolamine, replaced at 2 days by control medium. C shows culture in ethanolamine. Fresh medium added at 2 days. (Each line represents the mean of measurements on 5 explants.)

Measurements of fibroblasts and estimation of their volume were made according to a technic previously described (3). During the vacuolation process the cell volumes of four typical embryonic fibroblasts showed no changes. Strips of mouse muscle immersed in isotonic solutions with and without added ethanolamine (1 mgm. per cc. at pH 7.4) showed equal rates of gain in weight over the course of 8 hours. It therefore is probable that the vacuolation seen is not the result of imbibition of water from the surrounding medium.

In order to study the contents of these vacuoles an attempt was made to produce them in the giant ameba, *Chaos chaos*. Under the influence of toxic concentrations (about 0.5 to 1 mgm. per cc.) of ethanolamine motility ceased and the organisms ruptured, but without evidence of vacuole formation in the cytoplasm.

Cultures of chick embryo liver fibroblasts, after 2 days' growth in roller bottles, were treated with 1 mgm. per cc. of ethanolamine at pH 7.6, and after intervals of 2 to 12 hours were fixed and stained with hematoxylin in order to study the effects of the amine upon cells in mitosis. The only change noticed was an agglutination of the chromosomes into a central mass of chromatin. The chromosomes were not noticeably changed in length during this process.

The embryonic tissues studied were from various organs of chick, mouse, and human embryos. Fibroblasts derived from many tissues and epithelia from liver, iris, kidney, hypophysis, pancreas, and thyroid all showed characteristic vacuolation. Cultures of spleen and of blood cells failed to show vacuolation, although monocytes showed a reversible tendency to become rounded. Adult mouse and rabbit fibroblasts (Figs. 9 and 10) and adult rabbit kidney epithelium showed definite vacuolation, although in the latter tissue it was less intense than in the other normal tissues studied.

Relative ineffectiveness on malignant cells.—In contrast to the inhibition and vacuolation of normal cells, ethanolamine had relatively little effect *in vitro* on the fibroblasts of mouse sarcoma 180 or on the epithelium of a transplanted mammary carcinoma of the mouse. Cultures of tumors growing under conditions of ethanolamine concentration and pH which completely inhibit the growth of normal fibroblasts and epithelium are shown in Figs. 11 to 14. Inhibition of these tumors does not appear until four or five times the concentration of ethanolamine is used (at corresponding external hydrogen ion concentration) which inhibits normal cells (Fig. 1). Five roller bottle cultures of sarcoma and embryo tissue and 5 of carcinoma and embryo tissue were grown in ethanolamine, 1 to 3 mgm. per cc. at pH 7.6. In all cases only the malignant tissue grew.

Vacuolation has never been seen to occur in these tumors. When an inhibiting concentration is reached the cytoplasm of sarcoma 180 takes on a somewhat denser appearance than normal, while no alteration in the appearance of the carcinoma can be seen. These observations were first made in Carrel flask cultures, and were repeated in roller bottle cultures using a minimal amount of plasma, in order to ensure that the cells had adequate contact with the amine in solution.

Effects of other amines.—Following these observations a large series of compounds related to ethanolamine were studied in parallel experiments. The results of these experiments are summarized in Table I. In instances where inhibition is stated to be dependent upon pH, sufficient observations have been made to determine that this dependence is of the same type as in the case of ethanolamine; in other words, that the degree of inhibition follows in general the concentration of undissociated amine (RNH_2) in the medium,

and hence is much greater in relatively alkaline media. The degree of inhibition in each case has accordingly been observed at, or corrected by interpolation to, pH 7.4, and is expressed in molar terms. In addition, the dissociation constant (pK_B) has been cited in cases where a value is given in the literature, and from these two figures it has been possible to estimate the concentration of undissociated base (NH_2 groups) under conditions of threshold inhibition. In a few instances inhibition was not shown to bear the same quantitative relationship to pH, and in such cases the dependence upon pH has been stated as questionable.

From the results as shown in Table I it appears clear that virtually all of the aliphatic monoamines produce similar effects in approximately the same concentration (2-10 millimoles per liter). When the number of undissociated NH_2 groups is calculated, however, because some of the amines are stronger bases than others, it appears that growth inhibition cannot be considered dependent upon concentration of free amine in the medium, although such would appear to be the case with each individual amine under different conditions of concentration and pH (Table I).

Ammonia.—The case of ammonia is of some interest because its place in cell metabolism is better understood. Ammonia has never been observed to produce vacuolation of cells, as do the simpler amines. It is effective in higher dilution than the amines generally, and shows the same dependence upon pH for its inhibitory effects.

Amino acids.—A series of representative amino acids has been studied with results as shown in Table I. It will be obvious that all of them fail to show any of the characteristics of amine action. This is true as well of all three of the dibasic amino acids, which have an amine group in excess. All of the amino acids have been assayed in Tyrode solution, as it has been shown (18) that glycine is less inhibitory in the presence of serum (Fig. 7). The amino acids are clearly less inhibitory than the amines, although they have often been spoken of as inhibitors of growth. It requires 10 to 20 mgm. of glycine per cc. of medium to produce partial inhibition of growth, whereas 1 mgm. of methylamine per cc. inhibits it completely.

Other substances.—Ethylene glycol and dioxane were used in a few experiments, since they have been shown (11) to produce hydropic changes in kidney and liver cells suggesting the vacuolation caused by amines. No vacuolation of chick embryo kidney or liver was observed in inhibiting concentrations, as shown in Table I.

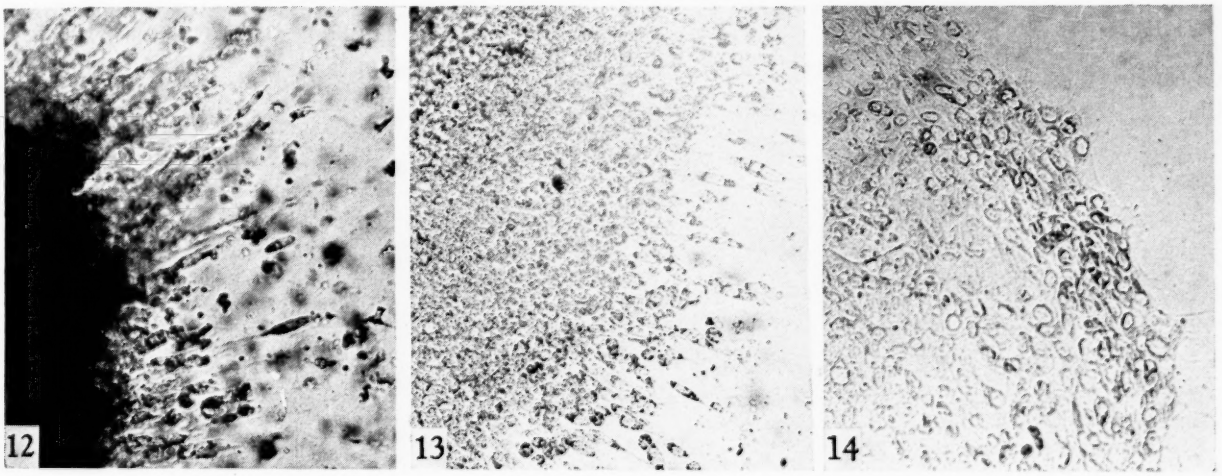

FIG. 12.—Two-day culture of mouse sarcoma 180 in ethanolamine 1:2,000, pH 7.4. Mag. × 100.
FIG. 13.—Three-day culture of mouse sarcoma 180 in ethanolamine 1:400, pH 7.5. Mag. × 70.
FIG. 14.—Five-day culture of mouse mammary carcinoma in ethanolamine 1:400, pH 7.4. Mag. × 100.

DISCUSSION

The correlation between pH of the medium and the concentration of any given amine required to produce a certain effect suggests at once that the concentration of undissociated amine (RNH_2) in the medium is the controlling factor. Under these circumstances, in accordance with the mass law equation involved, it might be expected that a typical amine would be effective at pH 8 in one-tenth the concentration required to produce the same effect at pH 7. This is true in the case of virtually all of the inhibitory amines studied, including ammonia, which produce vacuolation.

This cannot be taken to indicate, however, that the number of undissociated NH_2 groups in the medium is the sole factor producing vacuolation or inhibition, since similar concentrations of amines with apparently widely divergent dissociation constants produce similar effects, as in the case of mono-, di-, and triethanola-

TABLE I: PROPERTIES OF INHIBITING AMINES AND OTHER SUBSTANCES

Vacuolation produced	Dependence of inhibition upon pH	Approximate inhibitory concentration mil. eq./liter at pH 7.4	pK_B *	Inhibitory concentration of free NH_2 mil. eq./liter $\times 10^3$
Methylamine	+	3	3.36	1.7
Dimethylamine	+	3	3.29	1.5
Trimethylamine	+	2	4.20	8
Ethylamine	+	4	3.33	2.2
Diethylamine	+	7	3.02	1.8
Triethylamine	+	7	3.26	3.4
Tri-n-butylamine †	+	6	4.07	18
n-Amylamine	+	8	3.36	4.6
Iso-amylamine	+	8	3.36	4.6
Di-n-amylamine	+	4	2.82	0.7
Di-iso-amylamine	+	6	3.00	1.5
Ethanolamine	+	10	4.56	90
Ethanolamine phosphoric ester	?	15	...	...
Diethanolamine	+	8	5.12	260
Triethanolamine †	+	12	6.23	3,600
Propanolamine	+	8	...	...
Iso-propanolamine ‡	+	5	...	...
Tri-iso-propanolamine ‡	+	6	...	...
Acetylcholine	+	7	...	...
Morpholine ‡	+	40	5.70	4,400
Diethylaminoethanol ‡	+	32	...	...
Vacuolation not produced				
Ammonia	+	1.7	4.73	23
Ethylene diamine	?	165	4.07	480
Triethylene tetramine ‡	?	30	...	...
Choline	+	80	...	...
Betaine	+	80	...	...
Acetylethanolamine	..	>70	...	...
Trimethylamine oxide	..	>20	...	...
Phenyldiethanolamine ‡	..	30	...	...
Betanaphthylamine †	?	7	10.08	7,000
Amino acid mixture §	..	>50	...	...
Glycine	..	>150	...	...
Histidine	..	>10	...	...
Arginine	..	>10	...	...
Lysine	..	>10	...	...
Tyrosine	..	>10	...	...
Aspartic acid	..	>10	...	...
Tryptamine	..	>10	...	...
Histamine	+	10	...	...
Hydroxylamine	+	4	...	...
Hydrazine	+	6	...	...
Acetamide	..	>30	...	...
Ethylene glycol	..	>150	...	...
Dioxane	..	>40	...	...
Urethane	..	>20	...	...
Glucosamine	..	10	...	...
Urea	..	>40	...	...

* These values are drawn from various sources and may be subject to revision (10).
† Obtained through courtesy of Prof. L. F. Fieser and Wm. Doering.
‡ Kindly supplied by the National Carbide & Chemical Co.
§ Hydrolysate of Witte's peptone.

mines. We are at present undertaking a study of the correlation between biological and physicochemical properties of a few typical amines.

We have offered evidence that the vacuoles which appear under treatment by amines are aqueous, but that they are not caused by imbibition of water by the cell. They appear first as scattered vacuoles but when they have reached full size it appears as if the cytoplasm had become a reticulum of protoplasmic constituents (including, for example, the neutral red staining granules) containing aqueous lacunae. This reticular system appears to have greater rigidity than the normal protoplasm, as judged by the deformation of the nucleus which occurs. It seems likely that the phenomenon represents a redistribution of the aqueous and nonaqueous constituents of the cytoplasm, or a special form of gelation.

The vacuolar changes seen in these experiments are very similar to those observed in media lacking dextrose (13), and to those seen in high concentrations of potassium chloride (19). The vacuoles produced by *E. typhosa* (12) would appear to be of a different nature, since they are clustered around the nucleus and stain with neutral red.

The relative ineffectiveness of the amines in producing inhibition or other changes in tumor cells is of especial interest, since it is generally agreed (5, 7) that tumor cells are more susceptible than normal cells to a wide variety of deleterious influences *in vitro*.

If the assumption can be made (which is by no means certain) that the interior of the tumor cell is more acid (by pH 0.5) than that of the normal cell, this fact would suffice to explain a threefold difference in the threshold concentration. Such an assumption still does not afford a ready explanation of the failure of vacuolation to appear when inhibition does occur. This suggests a further distinguishing characteristic of tumor cells: either a metabolic peculiarity (*e.g.*, rapid destruction of the substances which produce vacuoles) or some important qualitative difference in the cytoplasm, or possibly failure of penetration of the amine into the cell.

As appears in Table I, certain groups in the molecule seem capable of suppressing the actions described; in particular, carbonyl, acetyl, and carboxyl groups do so almost uniformly. The carboxyl group, in fact, appears capable of suppressing both amino groups in the case of the dibasic amino acids. The notable exception to this rule appears in the case of choline and acetyl choline, in which it is the latter substance which produces vacuolation and the higher degree of inhibition.

The fact that primary, secondary, and tertiary amines are equally effective is of interest, since many of their chemical reactions are different. It is also of interest that those amines with more than one amino group, although as basic in properties as the mono-

amines, are ineffective. This might suggest that orientation of the molecules in surface films is an important factor in their inhibitory activity.

In an earlier paper (4), attention was called to the importance of considering whether inhibitors of growth *in vitro* might be of importance in the regulation of tissue growth in the living animal. It was suggested that those inhibitors which are of potential physiological importance must obey certain rules: they must be graded in their action, their action must be reversible, and they must appear in tissue in concentrations close to those which are effective *in vitro*. The first two criteria appear to be fulfilled in the case of the amines. In addition, the amines are able to inhibit normal tissue growth, while they do not inhibit the growth of tumor cells which, as a rule, are more readily damaged by most external influences. This is in striking analogy to the conditions usually existing in the tumor-bearing adult.

The remaining criterion, effective concentration in tissue, is more difficult to demonstrate. As we have cited above (17), doubt has been cast upon the existence of free ethanolamine or choline in living tissue. The phosphoric ester of ethanolamine has, however, been isolated from tumor (14) and intestine (6) in a concentration of about 0.4 mgm. per gm. of tissue, which is comparable to the amount causing inhibition of fibroblastic and epithelial growth.

The amino acids may probably be adjudged ineffective as far as growth inhibition *in vivo* is concerned. The same is true of urea. Ammonia, judging by its low concentration in the blood, is also probably unimportant. The status of the aliphatic amines generally remains in doubt. Although they are produced in large quantities by certain bacteria which decarboxylate amino acids, such a process in tissues of the higher animals seems highly improbable under normal conditions of life. The fact that none of the crude inhibitory fractions of liver show the specific characteristics of amine action (vacuolation and dependence upon pH) would make it appear unlikely that amines are the chief inhibitory constituents of liver extractives.

Summary

Most aliphatic amines (excluding diamines and amino acids) and the phosphoric ester of ethanolamine are inhibitory to growth of normal cells in culture in concentrations of about 3 to 15 millimoles per liter. The inhibitory effect shows a high degree of dependence upon pH of the medium in the case of most of these amines; in passing from pH 7 to pH 8, the effective concentration is reduced 90 per cent.

The amines produce an intense vacuolation of the cytoplasm within a few hours; this process consists in segregation of large aqueous lacunae surrounded by a protoplasmic reticulum. Both inhibition and vacuolation are reversible upon removal of the amine.

Sarcoma and carcinoma are much less susceptible to inhibition by amines than are normal cells, and will grow readily alongside wholly inhibited normal cells. Vacuolation does not occur in malignant cells even with high concentrations of amines.

The possibility that amines may play a part in the physiological regulation of growth is discussed.

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The Induction of Leukemia in Mice Following Percutaneous Application of 9,10-Dimethyl-1,2-Benzanthracene

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(Received for publication May 26, 1941)

Data regarding the influence of carcinogens on the incidence of leukoses in mice are relatively meager.

Morton and Mider (15, 16) were the first to show a definite influence of a carcinogen, methylcholanthrene, on the production of leukemia in a genetically controlled strain of mice, the Dba strain, which has a tendency to develop lymphoblastoma spontaneously. Previous to this report occasional leukoses had been observed in mice following treatment with several carcinogens (6, 13, 18), indicating some sort of relationship between carcinogen and leukemia. Brues and Marble (2) observed a decided increase in the incidence of lymphoblastoma in a derivative of the Bagg albino strain of mice following external application of tar, although this strain is particularly free from lymphomatoses. Likewise Furth and Furth (6) have described the production of a small number of neoplasms involving monocytic and myeloid cells although such lesions were rare in control mice. Furth and Barnes (7) report that subcutaneous and percutaneous application of carcinogens to mice of their Ak and Rf strains and hybrids between these resulted in an earlier appearance of leukemia. It was noted that in one strain of mice in which the typical spontaneous lesions were monocytic or myeloid the induced leukemias were all lymphoid. On the other hand it has been shown in the F strain of mice that painting with methylcholanthrene hastens the appearance of both the myeloid and lymphoid leukemias characteristic of the strain (8).

The present report deals with the effect of the potent carcinogen 9,10-dimethyl-1,2-benzanthracene [1] on the induction of leukemia in mice and a study of the morphology and transplantability of the induced leukemias.

Materials and Methods

Mice, 1 month of age, were painted twice weekly until tumors appeared with a 0.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene in thiophene-free benzene. The carcinogen was applied to the skin with a No. 4 camel's hair brush mid-dorsally from the interscapular to the sacral region.

Blood counts were made on mice showing a distinct enlargement of lymph nodes or on mice showing an enlarged spleen and liver. All white blood counts were made from cardiac blood. The cover slip method of obtaining differential counts was used and blood for these was obtained by cutting off the end of the tail. Sato's peroxidase method was used for differentiation of granules in the leukocytes. Splenic and lymph node imprints, as well as blood smears, were stained with Wright's stain.

Pieces of spleen, liver, lymph nodes, kidneys, and lung were fixed in Tellyesniczky's fluid, cut at 7μ, and stained in hematoxylin-eosin solution. These tissues from a few animals with characteristic leukemias were studied after fixation for 24 hours in 2 parts aqueous sublimate solution to 1 part alcohol and staining according to Giemsa's Azur-eosin process. The lactic acid-nitric acid method of decalcification was used for vertebral columns.

Transplants of leukemic and leukemic-like lesions were made with a sterile trocar. Approximately 1 cu. mm. of tissue was inoculated *sub cutem* into the right axillary region of the mice.

Reactions of the Dilute Brown, Dba Strain

The Dba strain of mice has been maintained for more than 25 generations of brother to sister matings. There exist at the present time four distinct sublines 1, 2, 3, and 4. All are sublines with high incidence of mammary carcinoma. Between 80 and 90 per cent of the breeding females develop breast cancer, while among virgin females the incidence is approximately 50 per cent. Lymphoblastoma occurs among both sexes but the incidence and type have not as yet been accurately determined. The appearance of breast tumors at a relatively early age (12) complicates an accurate determination of the incidence of leukoses which normally occur spontaneously somewhat later in life. Data obtained from ovariectomized mice show an incidence of nearly 30 per cent lymphoblastoma at a mean age of 480 days, whereas the incidence for females with unilateral ovariectomy is approximately 6 per cent with a similar mean age of appearance. Tumors of the liver, classified as reticulo-endothelioma,

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[1] Grateful acknowledgment is made to Dr. W. E. Bachmann, University of Michigan, Dr. L. F. Fieser, Harvard University, and Dr. M. S. Newman, Ohio State University, for supplying this carcinogen.

probably monocytic leukemia, occur in about 8 per cent of ovariectomized mice at a relatively later date.[2] Preliminary study of the types of leukoses occurring in the Dba strain has been made with Dr. G. W. Woolley. Lymphoid leukemia, myeloid leukemia, mediastinal lymphosarcoma, and a benign condition of extramedullary myelopoiesis are known to exist.

It has been shown previously that Dba mice react by the formation of a general lymphomatosis when painted with 9,10-dimethyl-1,2-benzanthracene (9). This study has been extended to include the reactions of 209 mice of this strain. In the first series (Table I) 119 mice of 5 different sublines have been painted with a 0.5 per cent solution of carcinogen. Three types of reactions were observed: lymphoid leukemia, which was usually expressed as an extreme general lymphomatosis (Figs. 1, 2, and 3), myeloid leukemia (Figs. 4 and 5), and a condition which appears to be monocytic leukemia (Figs. 6 and 7).

TABLE I: REACTION OF MONTH-OLD DBA MICE TO PERCUTANEOUS APPLICATION OF A 0.5 PER CENT SOLUTION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE

		Leukemia										
Subline	No. of mice	Lymphoid	Per cent lymphoid	Mean latent period,* days	Myeloid	Per cent myeloid	Mean latent period,* days	Monocytic	Per cent monocytic	Latent period,* days	Total leukemia	Per cent leukemia
D_1	29	24	81.0	111.8	3	10.0	113.0	1	3.8	113	28	97.0
D_2	23	20	86.9	103.3	1	4.4	165.0	..	...	...	21	91.3
D_3	18	17	94.4	100.5	..	...	....	..	...	...	17	94.4
D_4	19	14	73.7	101.8	3	15.8	92.3	1	5.3	105	18	94.7
D_T	30	26	86.6	114.8	1	3.3	183.0	..	...	...	27	90.0
Totals	119	101	84.9	107.6	8	6.7	120.5	2	1.7	114	111	93.3

* Latent period—time from initial application of carcinogen to appearance of lymphadenopathy or other symptoms of leukemia.

Lymphoid leukemia occurred in 101 mice of all sublines. The earliest lymphomatosis appeared at 45 days following painting. This lesion first appeared in the form of a general bilateral enlargement of the axillary, inguinal, and cervical lymph nodes. These nodes enlarged to 1 cm. or more and were soft and cellular. The spleen was greatly enlarged, pale, grayish, and nodular. The liver was usually enlarged and pale. In most cases there was involvement of the lumbar, renal, and mesenteric-intestinal nodes. Less frequently there occurred adenopathy of the mediastinum with accompanying hydrothorax. In the late stages of the disease there occurred extreme connective tissue edema and ascites. Macroscopically, invasion was noted in the lungs, kidneys, adrenal, skeletal muscle, pancreas, and ovaries. A typical protocol follows:

There appeared in ♀ D2005 143 days after initial painting with a 0.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene extreme bilateral enlargement of the axillary, cervical, and inguinal lymph nodes. The nodes were 1 cm. in diameter, soft, and grayish. The spleen was pale and nodular on the cut surface and measured 4.2 x 1.7 x 0.7 cm. The liver was greatly enlarged, pale, and nodular. There was extreme enlargement of the mesenteric-intestinal nodes, measuring 3 x 0.5 x 0.5 cm., and bilateral enlargement of the lumbar and renal nodes. Severe edema and anasarca occurred. Eight days later the animal was moribund and dyspneic and was killed. The white blood count was 123,600 per cu. mm. with 99 per cent of the cells medium-sized and large lymphocytes which were negative to the peroxidase test. Secondary anemia was severe. Histologically there was complete invasion of the lymph nodes with medium and large lymphocytes and infiltration of the capsules. The normal architecture of the spleen was entirely distorted with invasion of lymphocytes into the Malpighian corpuscles. Very little red pulp remained. In the liver there was extreme periportal and sinusoidal infiltration. Infiltration was also noted in the lungs and kidney. In the marrow spaces of the bone the atypical cells were numerous replacing for the most part the granulocytes.

This lesion has been transplanted through 10 generations with 100 per cent takes in mice of the same subline. A preliminary report on the influence of foster nursing on the growth of this tumor has been published (10).

There was great similarity among the induced lymphoid leukemias both as to clinical and histological features. In all cases there was involvement of the primary lymph nodes, liver, and spleen. In most cases there was extreme elevation of the white blood count and escape of lymphocytes into the blood. Two emaciated individuals, however, were leukopenic. Upon transmission, these tumors produced the characteristic features of the lesion with distinct elevation of the white blood count.

Twelve distinct lymphoid lesions were successfully transplanted and produced 100 per cent takes.

Myeloid leukemia was induced in 8 mice with a slightly later latent period. In contrast to a general lymphadenopathy mice of this group exhibited various symptoms. The abdomen was usually distended as a result of splenomegaly. One or more of the abdominal or mediastinal nodes showed adenopathy while the axillary, cervical, and inguinal nodes were generally slightly enlarged and often hemorrhagic. Kidneys and lungs were usually severely infiltrated. Ante-mortem blood counts showed severe anemia, an elevated white

[2] Unpublished data furnished by Dr. G. W. Woolley of this laboratory.

count, and the presence of numerous immature myeloid cells containing coarse granules in basophilic cytoplasm. A typical protocol follows:

Female D 17 appeared moribund and emaciated with a distended abdomen 172 days following the initial painting with carcinogen and was killed 2 days later *in extremis*. The spleen was greatly enlarged, pale and grayish, and nodular on surface. The liver was slightly enlarged and pale. The mediastinal and mesenteric-intestinal lymph nodes were greatly enlarged. All other nodes were slightly enlarged and some showed hemorrhagic spots. The kidneys appeared severely infiltrated. The cardiac leukocyte count showed 18,900 cells per cu. mm. The following differential count was obtained: lymphocytes 36 per cent, neutrophils 32 per cent, monocytes 3 per cent, eosinophils 2 per cent, and immature granulocytes 27 per cent with nearly equal distribution of myeloblasts and myelocytes, which were peroxidase positive. The architecture of the spleen was almost entirely obliterated with infiltration of immature and mature granulocytes in the red pulp. Megakaryocytes were noticeably increased. There was infiltration of mature and immature granulocytes in the sinusoids of the liver. Infiltration was noted in the affected lymph nodes. This was extreme chiefly in the medullary regions. Many plasma cells were present.

Five definite myeloid leukemias have been carried by transplantation with 100 per cent takes in the subline in which they arose. These produced the same characteristic histological features in the host animals as those which appeared in the induced lesions.

The third type of reaction evoked in this series as well as in older treated mice involved cells resembling histiocytes. The round form of the malignant cells appeared in the circulating blood. These were considerably larger than the normal monocytes of the blood and had very large bean-shaped nuclei. A typical case is as follows:

Ninety-five days following initial painting ♀ D600 showed extreme abdominal swelling, was moribund and dyspneic. The spleen was enlarged to 3 x 0.7 x 0.4 cm. It was pale and slightly nodular on the cut surface. There was extreme enlargement of the liver which showed numerous large grayish nodules and some areas of hemorrhage. Extreme adenopathy of the mesenteric-intestinal lymph nodes was observed. No other nodes were involved. Severe anasarca occurred. There was only slight elevation of the leukocyte count and numerous monocytes and neutrophils were present. Histologically the following features were observed. Part of the splenic architecture was preserved but there was infiltration of granulocytes in some regions. One large region in the red pulp at the base of a Malpighian corpuscle showed infiltration by large nongranular cells. These had abundant pink-staining cytoplasm. Some of these large round cells apparently invaded the Malpighian corpuscle. In the liver the sinusoids were packed with these cells. Here they were chiefly elongated and the nuclei assumed various shapes and forms. Large cells resembling those seen in the spleen were present in the medulla and sinuses of the mesenteric-intestinal lymph node.

These neoplastic changes have been carried through 8 transplant generations. There is little or no growth of the tumor subcutaneously but extreme systemic invasion occurs with an elevation of the leukocyte count and escape of monocytes into the blood stream.

Comparative and differential blood counts of the induced leukemias are given in Table II.

TABLE II: COMPARATIVE AND DIFFERENTIAL BLOOD COUNTS OF EXPERIMENTAL LEUKEMIC MICE OF THE DBA STRAIN *

	No. of mice	Wbc. per cu. mm.	Differential count in percentages					
			Lymphocytes	Neutrophils	Monocytes	Eosinophils	Myeloblasts	Myelocytes
Lymphoid leukemia	17	70,990	91.3	5.7	0.6	0.4	2.0	0.0
		(1,000-252,500)	(66-99)	(1-20)	(0-3)	(0-3)	(0-10)	
Myeloid leukemia	6	36,180	32.0	32.0	2.6	0.5	14.7	10.7
		(7,800-80,700)	(14-60)	(2-64)	(1-5)	(0-2)	(10-20)	(4-21)
Monocytic leukemia	3	116,750	22.0	53.0	21.5	0.0	2.0	1.5
		(37,850-221,400)	(20-26)	(43-66)	(14-29)		(1-3)	(0-4)
Controls	7	3,960	66.0	24.0	8.0	2.0	0.0	0.0
		(2,900-5,400)	(62-71)	(18-26)	(3-21)	(0-3)		

* The ranges of values are given in parentheses below the averages.

FACTORS AFFECTING CARCINOGENESIS

a) Sex. There was no influence of sex on the incidence of induced lymphoid leukemia or on the length

DESCRIPTION OF FIGURES 1 TO 7

FIG. 1.—Dba mouse showing generalized lymphomatosis, with enlargement of axillary, cervical, inguinal, and lumbar lymph nodes.

FIG. 2.—Liver, showing infiltration in generalized lymphomatosis. Mag. × 200.

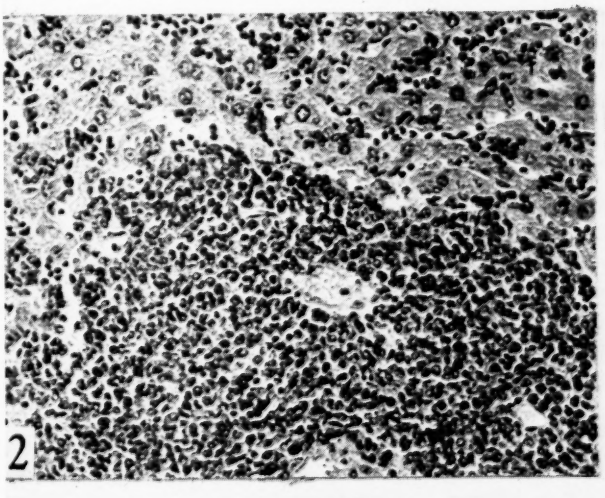
FIG. 3.—Axillary lymph node in generalized lymphomatosis. Complete obliteration of normal architecture by infiltrating lymphocytes. Mag. × 25.

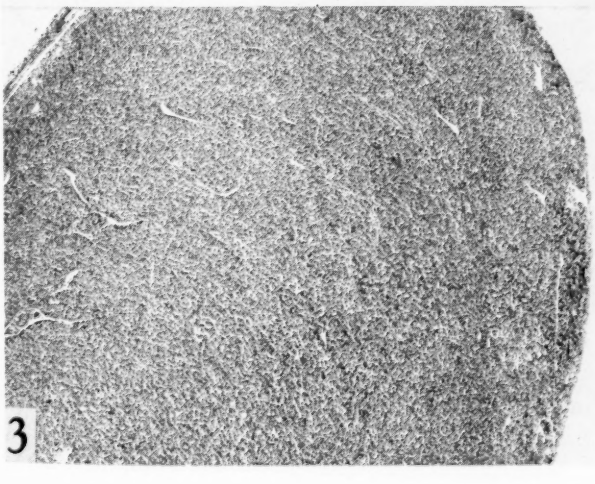
FIG. 4.—Sinusoidal infiltration of immature granulocytes in liver of Dba mouse with induced myeloid leukemia. Mag. × 200.

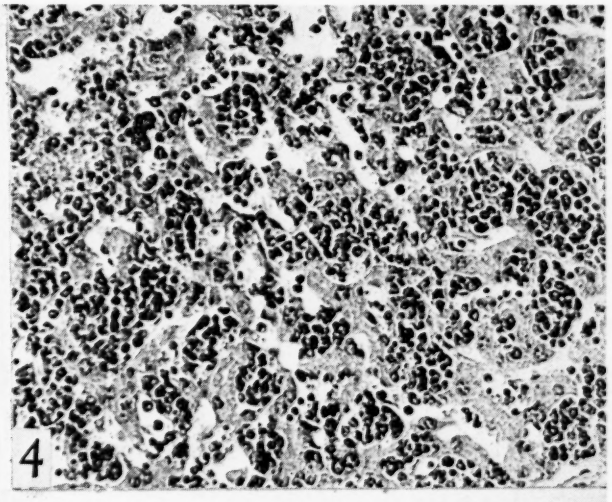
FIG. 5.—Region of spleen of C strain mouse with benign extramedullary myelopoiesis showing immature and mature granulocytes. Mag. × 600.

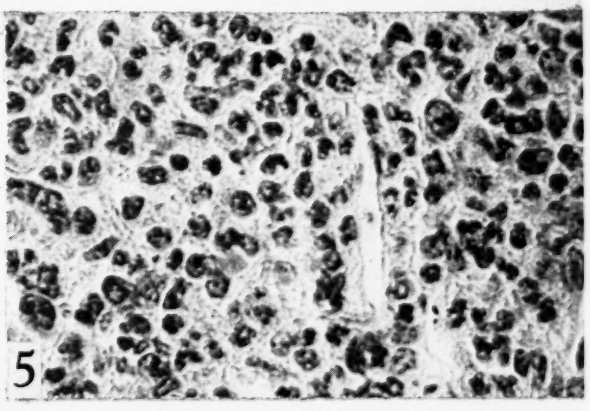
FIG. 6.—Liver of mouse showing monocytic infiltration. Mag. × 200.

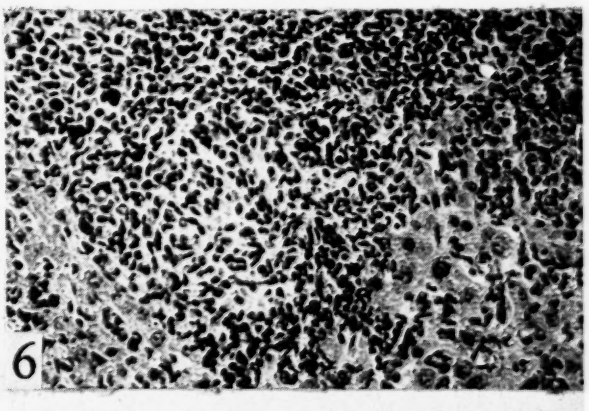
FIG. 7.—Blood smear of a Dba mouse showing monocytic leukemia. Mag. × 600.

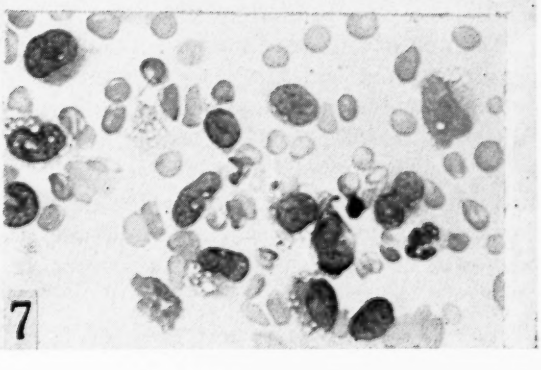












FIGS. 1-7

of the latent period. Among the treated females 85 per cent developed leukemia. The mean latent period was 106 (45-154) days. Among the males 83.8 per cent showed lymphoid leukemia 109.7 (63-200) days following painting.

b) Dosage. Only 3 of 15 mice painted twice weekly with a 0.1 per cent solution of 9,10-dimethyl-1,2-benzanthracene showed general lymphomatosis. Dosages greater than 0.5 per cent were rather toxic resulting in severe skin ulcerations in some cases. Subcutaneous injections were less effective. Injections of 0.3 mgm. (near subtoxic dose) of the carcinogen in olive oil induced only 10 per cent (3 out of 30) lymphoid reactions at a relatively later age.

c) Subline. Of the 4 sublines of the Dba strain existing at this laboratory, subline 1 has the lowest incidence of lymphoblastoma and subline 2 has a much higher incidence. Sublines 3 and 4 apparently are more like subline 2. Although there exists this difference in the spontaneous incidence of leukoses, there was found no significant difference in the reaction of the 4 strains (see Table I). Descendants of transferred ova Dba mice, D_T subline,[3] reacted in a manner similar to normal mice, although transferred ova mice apparently are affected by foreign uterine influences as measured by their spontaneous tumor reaction (5) and by their behavior to transplantable tumors (4).

d) Age. The influence of age of the animal upon the induction of tumors by carcinogens has not as yet been established. Opposite conclusions have been reached from experimental results (19). As regards the induction of leukoses in young as compared with old mice, Morton and Mider (16) report that there was a slight prolongation of the latent period of methylcholanthrene-induced leukoses in the older Dba mice of the age groups 81-100 days and 101+ days. Furth and Barnes (7) suggest that leukemia is induced following percutaneous application of carcinogen more readily in mice approximately 3 weeks of age than in those 5 to 7 weeks of age.

[3] These mice constitute the F_1 to F_9 generations of Dba mice developed from ova transferred to uteri of mice of the C57 black inbred strain. They were obtained from Miss Elizabeth Fekete.

Forty-five Dba mice of ages ranging from 2+ to 16 months were treated similarly to the series of mice one month of age. Sexes were evenly distributed and although a few of the mice developed breast tumors, only those are considered which lived beyond the mean age of death of the younger mice. Only 15.5 per cent of these mice developed a general lymphomatosis with a mean latent period of 122 days. One myeloid leukemia and 3 monocytic leukemias appeared. The majority of mice, 11, or 40 per cent, developed a benign condition of extreme myeloid hyperplasia similar to the spontaneous condition described by Barnes and Sisman (1) and called extramedullary myelopoiesis. The mean latent period for this lesion was 155 days (Table III).

This condition appeared as an abdominal swelling with no apparent involvement of the subcutaneous lymph nodes. Mice with this lesion lived considerably longer than those with leukemic reactions. The spleen and liver were chiefly involved. Extreme splenomegaly was always present and the spleen was grayish and nodular. The mesenteric-intestinal lymph node in some cases showed extreme enlargement. Generally there was elevation of the white blood count with a change of the normally existing lymphocytosis in mice to a condition of neutrophilia with very few or no immature granulocytes appearing in the peripheral blood. Histologically there was pronounced myeloid hyperplasia of the spleen. The red pulp was infiltrated with mature and immature granulocytes, chiefly metamyelocytes. Malpighian corpuscles were greatly compressed and myeloid cells were not noted in these centers. There were numerous megakaryocytes and regions of erythropoiesis throughout the red pulp.

Infiltration of mature and immature granulocytes was not severe in the liver and when present was mainly sinusoidal. Infiltration in the mesenteric-intestinal lymph nodes occurred chiefly in the medulla and no encapsular infiltration was noted. There were many plasma cells in the sinuses of the node. Foci of infiltration or myelopoiesis have been seen infrequently in the kidney and lung. Maturation was conspicuous

TABLE III: REACTION OF OLD MICE OF THE DBA STRAIN TO PERCUTANEOUS APPLICATION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE

Age of mice	No. of mice	Reaction					
		Lymphoid leukemia	Myeloid leukemia	Monocytic leukemia	Per cent leukemia	Extramedullary myelopoiesis	Per cent myelopoiesis
2+ mo.	5	1	..	1	40.0	1	20.0
4 mo.	2	..	..	..	...	1	50.0
7 mo.	4	1	1	..	50.0	1	25.0
8 mo.	13	2	..	..	15.4	5	38.4
9 mo.	10	1	..	..	10.0	5	50.0
12 mo.	5	..	..	2	40.0	2	40.0
16 mo.	6	2	..	..	33.3	3	50.0
Total	45	7	1	3	24.4	18	40.0

in the bone marrow. Attempts at transmission of six of these lesions were unsuccessful.

Although many of the painted mice developed papillomas and carcinomas, the presence of skin tumors was not a necessary antecedent for the development of extramedullary myelopoiesis since some mice without skin neoplasms developed this lesion. Likewise some mice with rapidly growing carcinomas failed to develop extramedullary myelopoiesis.

It seems improbable that these lesions are merely accentuations of changes sometimes appearing following treatment with carcinogens since only 4 of 67 control mice similarly painted with the carcinogens phenanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene and an irritant, acetic acid, showed a slight spleen involvement in the direction of myeloid hyperplasia.

The decrease in incidence of induced lymphomatosis in old mice as well as the lengthening of the latent period suggests the development of some type of resistance. A similar resistance has been noted to influence the latent period for transplantable leukoses.

Reactions of the Bagg-MacDowell C Strain Mice

Mice of the C strain have been maintained by brother to sister matings for over 45 generations. This strain was obtained at the 25th generation of inbreeding by Dr. G. D. Snell from Dr. E. C. MacDowell and has been carried for 20 generations at this laboratory. Complete data about tumors are not available. It is known, however, that there is a relatively low incidence of mammary carcinoma (1 to 5 per cent), a relatively high incidence of pulmonary carcinoma, and a fairly high incidence ($\pm$50 per cent) of lesions involving the liver and spleen, some of which are known to be definitely leukemic.

Four types of reactions appeared among the 44 mice painted with 9,10-dimethyl-1,2-benzanthracene. Involvement of lymphoid tissue occurred in 5 animals. Three, 7.5 per cent, showed distinct general lymphomatosis similar to that appearing in the Dba strain (average latent period, 117.7 days). There was extreme lymphocytosis of medium and large cells which were peroxidase negative. Infiltrations were present in lymph nodes, spleen, liver, lung, kidneys, and ovaries. Two mice, 4.5 per cent, developed severe mediastinal lymphosarcoma with accompanying hydrothorax (average latent period, 162 days), but no infiltration was noted in other organs.

Thirty-three of the experimental mice, 75 per cent, showed lesions involving myeloid elements. Five, 11.4 per cent, have been classified as myeloid leukemia (average latent period 139.6 days) and 28, 63.6 per cent, as the benign condition extramedullary myelopoiesis (average latent period 175.4 days). The distinction between the myeloid disturbances was made by the following criteria. Transplantation was successful in the myeloid leukemias attempted. No success was attained in 6 attempts at transfer of the extramedullary myelopoiesis lesions. Leukemic animals exhibited involvement of many of the lymph nodes as well as nonblood-forming organs; *i.e.,* lung and kidney. In many cases hemorrhage was present in the lungs and nodes. The majority of the cells infiltrating the spleen and liver were immature, chiefly of the myeloblast group in the leukemic mice, and the liver in most cases showed extreme periportal and sinusoidal infiltration with these cells. Erythrogenic foci and megakaryocytes were more numerous in mice with extramedullary myelopoiesis. Although in both types of disturbance there was extreme leukocytosis, many immature myeloid cells were present in the blood of leukemic mice and very few or none were found in the peripheral blood of the nonmalignant condition.

In this strain numerous epitheliomas appeared at approximately 4 months following initial painting with carcinogen. In one animal, ♀ C 30, 154 days following initial painting an ulcerating epithelioma appeared coincident with a severe myeloid disturbance. The axillary and cervical lymph nodes showed moderate adenopathy. There was extreme splenomegaly and involvement of the mesenteric-intestinal lymph node. The liver appeared normal. Tissue from the axillary node was transplanted to mice of the C strain. Huge subcutaneous masses appeared in a short time. At 30 days there was splenomegaly, liver involvement, and enlargement of the subcutaneous lymph nodes of recipient mice.

Histological examination of the axillary lymph node used in transplantation revealed that the medulla was filled with metastatic epithelioma cells. Coexisting in the medulla and in the sinuses of the node were mature and immature cells of the myeloid series. This epithelioma was carried through 5 transplant generations. A coincident myeloid hyperplasia occurred in the 20 mice which grew the tumor. Four mice failed to grow epitheliomas and in these there was no myeloid hyperplasia. The blood counts of mice bearing the transplanted tumors and of normal control mice are given in Table IV.

According to the criteria used to distinguish between myeloid leukemia and extramedullary myelopoiesis, this condition, induced in a C strain mouse following painting with 9,10-dimethyl-1,2-benzanthracene, coincident with and transmissible by an epithelioma is not leukemia but nonmalignant extramedullary myelopoiesis.

Transplants of other epitheliomas induced by 9,10-dimethyl-1,2-benzanthracene and by cholanthrene and 5-methylcholanthrene in the C strain have not pro-

duced or been accompanied by any marked myeloid disturbance.

Parsons (17) and Burrows and Cook (3) report that sarcomas induced by the water-soluble sodium 1,2,5,6-dibenzanthracene-9-10-endo-α,β-succinate when transplanted to normal mice brought about a leukemic condition.

The apparently benign lesions of extramedullary myelopoiesis observed in the C strain were similar to those appearing in painted old Dba mice. Whether this is a constitutional effect resulting from painting with the carcinogen or whether the condition is a direct result of the carcinogenesis is not known. Constitutional effects of tars and known carcinogens have been studied with reports of leukopenia or lymphopenia on

Table IV: Blood Counts of C Strain Mice Which Grew Induced Epithelioma

No. Mouse	Wbc. per cu. mm.	Differential count in percentages					
		Lymphocytes	Neutrophils	Monocytes	Eosinophils	Myeloblasts	Myelocytes
C 200	20,500	13	80	0	3.0	0	4
C 201	48,800	17	59	0	2.0	5	17
C 202	48,000	16	69	0	0.0	2	13
C 219	3,600	25	39	0	0.0	7	29
C 221	34,000	16	74	0	1.0	3	6
C 222	25,800	18	70	0	1.0	1	10
C 230	45,400	16	65	0	1.0	2	16
Mean values:							
Experimental—7 mice	32,300	17	65	0	1.0	3	14
Control—9 mice	3,920	63	23	13.5	0.5	0	0

Table V: Reactions of Other Strains of Mice Painted with 9,10-Dimethyl-1,2-Benzanthracene

Strain	No. painted	Period of painting, days	Reaction			
			Lymphomatosis	Myelopoiesis	Tumor—skin	Tumor—lung
Leaden	20	150	0	0	15	0
C3H	22	120	0	3	21	0
Swiss	15	150	12	0	4	0
ABC	15	150	0	2	10	0
N	12	150	0	0	7	0
C57 black	30	120	0	7	15	2
C57 brown	30	100	0	3	21	2

the one hand and myeloid hyperplasia on the other. Twenty control C strain mice painted with a 0.5 per cent solution of 1,2,5,6-dibenzanthracene did not show a general myeloid hyperplasia. Likewise not a single case of myeloid hyperplasia was noted histologically among 45 C strain mice inoculated *sub cutem* with 0.8 mgm. 1,2,5,6-dibenzanthracene in olive oil although 77 per cent of these developed large, fast growing sarcomas and lived to a mean age of 253 days.

Reactions of Other Strains of Mice

Of seven other strains of mice similarly treated with 9,10-dimethyl-1,2-benzanthracene, only one, the Swiss strain, has reacted in the development of a definite leukosis. Twelve of 15 Swiss mice developed a severe general lymphomatosis at a mean latent period of 133 days. This condition was histologically identical with that appearing in the Dba strain. Four of these lymphomatoses have been successfully transplanted. The reaction in this strain was unexpected since no spontaneous lesions involving the lymphatic system have been observed. Early appearance of carcinomas of the skin in the C3H, C57 brown, and C57 black strains may have lessened the opportunity for true leukoses to appear (Table V).

Summary and Conclusions

Three types of neoplastic reaction have been observed in mice of the Dba strain following percutaneous application of the carcinogen 9,10-dimethyl-1,2-benzanthracene to month-old mice. A general lymphomatosis (lymphoid leukemia) occurred in 101 of 119 mice (84.9 per cent). The mean latent period was 107.6 days. The earliest lesion appeared 45 days following initial painting. Myeloid leukemia occurred in 8 mice (6.7 per cent) and monocytic leukemia in 2 mice (1.7 per cent). Thus, following treatment with the carcinogen, a decisive increase of lymphoid leukemia resulted in experimental Dba mice and these lesions appeared much earlier. Among control mice the maximum incidence observed for all leukemias was 30 per cent and these occurred at an average age of 480 days.

As regards the induction of myeloid leukemia and monocytic leukemia, there was apparently no increase in the incidence of these lesions but their appearance was considerably hastened.

The optimal dosage of carcinogen required for induction of leukemia following painting was 0.5 per cent. Subcutaneous injections of subtoxic doses were less effective.

No influence of the factors of sex, subline, or foreign uterine stimuli on the incidence of induced leukoses was found. Mice of the Dba strain which were from 2+ to 16 months of age at the initial painting were significantly less susceptible to the leukogenic action of the carcinogen than month-old mice. The chief reaction observed in older mice was a benign extramedullary myelopoiesis.

Following similar treatment with 9,10-dimethyl-1,2-benzanthracene, lymphoid lesions resulted in only 5 of 44 Macdowell-Bagg C strain mice (7.5 per cent). Three of these lesions were of the definite generalized lymphomatosis type and 2 were mediastinal lymphosarcomas. The majority of the reactions involved the myeloid elements of the blood-forming organs. Five cases of myeloid leukemia and 28 cases of benign extramedullary myelopoiesis were observed. The distinctions between these two types of disturbances are discussed.

A condition of extramedullary myelopoiesis which appeared coincident with an induced epithelioma and which was also transmissible by an induced epithelioma is discussed.

Seven other inbred strains of mice were painted with a 0.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene. Only one, the Swiss strain, reacted with the formation of a generalized lymphomatosis.

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A Genetic Analysis of the Induction of Tumors by Methylcholanthrene

II. The Influence of Spindle Cell Sarcoma and of Carcinoma of the Skin upon Each Other*†

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(Received for publication May 7, 1941)

Data have been presented which showed that a variety of tumors could be induced by methylcholanthrene when injected into a series of hybrid mice of the NH descent (11). The tendency to give rise to specific types of tumors is undoubtedly inherited since it is transmitted to the lineal descendants, thus giving rise to distinct sublines. These sublines were established by the genetic principle of using for breeders only the lineal descendants of those mice which conformed to type (progeny test). In the course of the establishment of these various sublines which gave specific types of tumors at different latent periods, an opportunity for studying the frequency distributions, possible influence of one type of tumor upon another, and several other problems of general interest has been made possible.

The present analysis is based upon observations on 800 NH mice, kept on a diet of Nurishmix. All mice were weaned at 30 days of life. At 60 days of life they received subcutaneously 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. All mice were examined once a week. All observations were charted on coordinate paper. As soon as any palpable mass began to grow progressively the animal bearing it was killed. All tissues were fixed in Bouin's fixative and stained with hemotoxylin and eosin. Of these 800 mice, 592 developed tumors either locally or at sites remote from the point of injection. Two hundred and nine developed spindle cell sarcoma; 83, carcinoma of the skin; 56, mixed tumors involving both carcinoma of the skin and spindle cell sarcoma; and 244 mice developed a variety of tumors, either singly or in combination, such as adenocarcinoma of the mammary gland, carcinoma of the lung, and rhabdomyosarcoma. These latter types will be discussed in separate papers.

In this paper only data on the frequency distribution of (a) the total number of induced tumors irrespective of type, (b) spindle cell sarcoma, (c) carcinoma of the skin, and (d) mixed tumors showing both the presence of carcinoma of the skin and spindle cell sarcoma in the same tumor mass are included. These data are given in Fig. 1.

From an examination of Fig. 1 it may be seen that (a) carcinoma of the skin occurs early, (b) spindle cell sarcoma occurs after a relatively greater latent period, and (c) when carcinoma of the skin and spindle cell sarcoma are present simultaneously in the same tumor-bearing mouse, the same frequency distribution of age is obtained as that obtained when carcinoma of the skin is present alone.

Discussion

In 1865, Thiersch (12) expressed the opinion that in the origin of carcinoma there is a disturbance in the static equilibrium which normally exists between the epithelium and connective tissue, thus allowing the penetration of the epithelium into the underlying tissues, such infiltration being favored by the "hypothetical" softening of the stroma occurring in old age. Many criticisms of this conception have, from time to time, been advocated; for example, (a) the occasional occurrence of carcinomas among young people and (b) the connective tissues themselves form sarcoma (5).

Many attempts have been made to show a possible correlation between susceptibility to tumors induced by one of the many carcinogens and genetic susceptibility to spontaneous tumors as found in mice in the various inbred strains. Kreyberg (6, 7) pointed out that the genetic or constitutional factor had been established primarily for carcinoma of the mammary gland. Consequently there was no correlation between

* This investigation was aided by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

† Read at the 34th Annual Meeting, American Association for Cancer Research, Inc., Chicago, Ill., April 15, 1941.

natural susceptibility to carcinoma of the mammary gland and susceptibility to the induction of spindle cell sarcoma by a carcinogen.

On the other hand, Lynch (8-10) has shown very convincingly that a definite correlation exists between susceptibility to induction of lung tumors by a carcinogen and the natural or genetic susceptibility to the development of spontaneous carcinoma of the lung in mice. This finding has been verified by Andervont (1). Andervont (2) has also studied the occurrence of spontaneous tumors of the lung and only an occasional spontaneous carcinoma of the lung. That they develop a multiplicity of tumors when subjected to the same amount of methylcholanthrene is more than likely due to the high degree of biological variability originally produced in them by hybridization, which apparently does not manifest itself in the development of spontaneous neoplasia.

Perhaps a similar situation is the one reported by Gardner and Strong (4) in which they find that the response to estrogen in C57 mice manifests itself in the form of pituitary adenomas. This response is

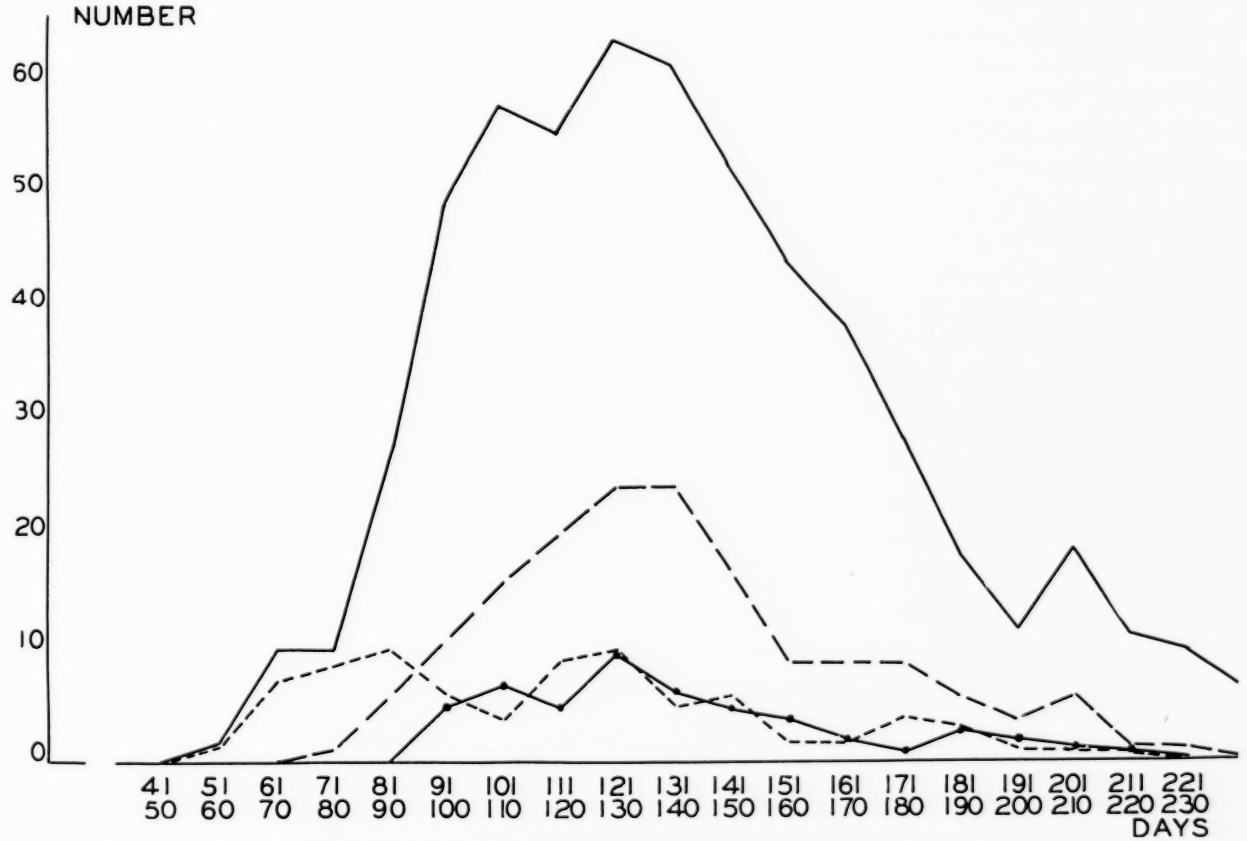

FIG. 1.—The frequency distribution of (a) total number of methylcholanthrene-induced tumors in mice of the NH strain, 592 tumors—solid line, (b) spindle cell sarcoma, 209 tumors—long dash line, (c) carcinoma of the skin, 83 tumors—short dash line, and (d) mixed tumors involving both carcinoma of the skin and spindle cell sarcoma, 56 cases—dot and solid line. Age in days is plotted along the base line; number of mice is given on the vertical line.

mammary gland and induced subcutaneous and pulmonary tumors in 8 inbred strains. He concludes that there is no correlation between the susceptibility to spontaneous mammary tumors and susceptibility to the induction of subcutaneous growths. Recently Burdette and Strong (3) published data similar to Andervont's, indicating differences in the latent periods between mice of the various inbred strains, but whether these differences are the resultant of a general factor of susceptibility to cancer is by no means clear.

Mice of the NH strain which served as controls for the animals tested with methylcholanthrene in this experiment have up to the present time shown apparently inherited as a dominant but so far this "genic complex" has never manifested itself in the development of spontaneous pituitary adenomas or of any other type of neoplasm. The phenomenon reported here is apparently not similar to the induction of carcinoma of the mammary gland by estrogens in male mice belonging to strains in which the females show a high incidence of carcinoma of the mammary gland (strain-limited response). Whether one or more mechanisms are involved in the spontaneous origin and induction of tumors by one of several means is not yet clear.

The data presented in this paper, showing that

after a latent period of 91 days the age distribution of mixed tumors, carcinoma of the skin, and spindle cell sarcoma is the same as for carcinoma of the skin alone, would indicate that the presence of spindle cell sarcoma has had no detectable influence on the origin of carcinoma of the skin and vice versa. This suggests that the two neoplastic conditions are probably entirely independent of each other. Since neither type ever occurs spontaneously in mice of the NH strain, there is no possibility of drawing a correlation between spontaneous susceptibility and induced susceptibility as has been so successfully demonstrated in carcinomas of the lung by Lynch (8-10) and by Andervont (1), and in leukemia by Kirschbaum, Strong, and Gardner (5). These particular types of induced tumors occurring both alone and together in different individuals are probably brought about by the peculiar biological variability of individuals in response to a carcinogen. The latent period or time during which the carcinogen acts is also a very important factor in determining the type of tumor finally induced, but this also is probably merely a measure of intrinsic constitution.

Summary and Conclusions

1. Carcinoma of the skin is the earliest type of tumor induced in NH mice by the injection of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil and injected subcutaneously at 60 days of life.

2. The latent period for the induction of spindle cell sarcoma by the same technic is longer than it is for carcinoma of the skin.

3. Both carcinoma of the skin and spindle cell sarcoma frequently occur together in the same tumor mass and when they do the latent period and the frequency distribution of either type is not disturbed by the presence of the other type.

4. The origins of carcinoma of the skin and of spindle cell sarcoma are apparently independent of each other and are probably brought about by a peculiar biological constitution of the individual which gave rise to them.

Dr. Robert Tennant has very kindly diagnosed many of the sections.

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Sex Difference in the Conjunctive Tumoral Reaction of the Guinea Pig Towards Natural and Artificial Estrogens*

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Introduction

The conjunctive tumoral reaction of the male guinea pig towards esterified estradiol is different from that of the female (7).

Large spherical peritoneal fibroids are easily elicited in the female guinea pig injected thrice weekly with 80 μgm. or less of estradiol [1] given as the benzoate in the course of 2 to 3 months (6, 9, 10). Similar fibroids are not induced in the male under similar conditions. The tumoral seed on the spleen, on the surrounding parts of the abdominal wall, and on the ventral surface of the stomach, and peritoneal fibrous strands are the characteristic features of the conjunctive reaction in the male (6, 7). In the male the localization of the reaction on the left side is of great uniformity, especially at the beginning (19). In contrast, the variation is enormous in the female (5, 9, 10, 21). The reaction in the male is probably due to a lesser susceptibility. This is suggested by different facts. The left-sided tumoral seed typical of the male is found frequently in females treated with smaller quantities of estrogens (1, 15). An identical feature is observed in the female when the tumorigenic action of the follicular hormone is inhibited by other hormones such as testosterone, progesterone, or desoxycorticosterone (12, 13). Individual spherical fibroids appear also in the male when the injections of 80 μgm. of estradiol benzoate are prolonged for more than 4 months (7) or when similar quantities of the 17-caprylate or the 17-benzoate-3-n-butyrate ester of estradiol (5) which possess more potent tumorigenic power than the benzoic or dipropionic esters of estradiol (8) are used. And finally, the uniformity of the reaction in the male becomes highly accentuated when a smaller dose of the 17-caprylate as, for instance, 10 μgm. per injection is used (2), a dosage which in the female is sufficient to elicit large abdominal fibroids (6). The sex difference of the conjunctive reaction was quite evident also in experiments in which tablets of free estradiol were used instead of injections of esterified estradiol (3).

The above facts have been established by our group on a number of animals sufficiently large to reduce possibility of error. Seventy-eight male and several hundred female guinea pigs were injected in the course of 69 to 140 days (2, 6, 7, 19) or treated with subcutaneous tablets (3). Full corroboration of our findings concerning a sex difference in the conjunctive tumoral reaction has been published by other workers (17).

The endocrine activity of the gonads is not responsible for this sex difference. We have reported that the castrated male guinea pig reacts in a way similar to the noncastrated male (6, 7). One must then question whether there is a sex difference in the metabolic fate of the follicular hormones which might explain the sex difference in the tumoral reaction to estrogens. Comparative experiments with natural and artificial estrogens offer an opportunity to study this question experimentally. Artificial estrogens are more resistant against inactivation than the natural follicular hormones (4, 18). Therefore, there should be some difference between the metabolic behavior in the organism of artificial and natural estrogens. If the sex difference in the conjunctive reaction towards follicular hormones as established by our experiments were due to some unknown sex difference in the metabolic fate of the hormones, one might question whether male or female guinea pigs would also react towards artificial estrogens in a different manner according to sex, as they do towards natural estrogens.

Experiments [2]

Thirty-seven male guinea pigs, weighing 235 to 680 gm., were injected with doses of stilbestrol or hexestrol which in the female are sufficient to elicit abdominal fibroids, or with doses four times greater. The results in the males were compared with those obtained in 23 castrated females. The artificial estrogens were given in olive oil; 0.4 cc. containing 80 or 320 μgm. of stilbestrol, and 40 or 160 μgm. of hexestrol respectively, were injected thrice weekly in the course of 87 and 88 days (with the exception of 3 animals injected for 73 to 84 days only.) A total of 38 injections (3 to 12 mgm. of stilbestrol, and 1.5 to 6.3 gm. of hexestrol) was given. These timing conditions corresponded to those in our former work with the injection of free or esterified follicular hormones into males (6, 7).

The results are summarized in Table I in which is given the number of animals which have developed

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, from Mr. Adolfo Eastman of Limache (Chile) and others.

[1] The artificial estrogens were generously supplied by Professor E. C. Dodds of the Courtauld Institute of Biochemistry, The Middlesex Hospital, University of London.

[2] See thesis of J. Palma (16).

extragenital tumors of a certain size; *i.e.*, "class 1," or tumors of a diameter not less than 1 mm., according to our classification (8, 11). It is evident from Table I that 38 injections of 80 μgm. of stilbestrol or of 40 μgm. of hexestrol given in the course of 3 months are unable to elicit tumors in the male whereas with the same treatment a high incidence of extragenital fibroids occurs in the female. Tumors appear in the male when quantities four times greater are given (Fig. 6). Even then the frequency of tumors in the male is very small when compared with that in females treated with the same quantity or with quantities four times smaller. With 320 μgm. of stilbestrol or with 160 μgm. of hexestrol per injection there was only one animal with individual spherical tumors of class 1 or larger. Fig. 6 shows the condition of the only male with tumors in the group injected with 320 μgm. of stilbestrol. The reaction in the only animal with tumors in the group receiving 160 μgm. of hexestrol was less pronounced than this.

As we observed in former experiments with natural estrogens, absence of individual spherical tumors in the male did not exclude other types of conjunctive proliferation. All the 37 males with but one exception showed some sign of a conjunctive reaction. The results are summarized in Table II. There was in most of the animals a "tumoral seed"; *i.e.*, an accumulation of small white nodules on the surface of different organs. The localization of these nodules whose microscopical structure we have described (14), was by preference on the left side—on the spleen, on the ventral surface of the stomach near the great curvature, on the abdominal wall and diaphragm in the vicinity of the spleen (Figs. 2 and 5). This left-sided orientation of the tumoral seed on the stomach and abdominal wall may be present when the tumoral seed on the spleen is lacking. In one animal injected with 320 μgm. of stilbestrol per injection the tumoral seed was found also on the intestine, especially on the cecum and ascending colon (Fig. 6). This was the only animal in which large tumors were present. The tumoral seed on the intestine is a very rare localization. In our previous experiments it occurred only after injection of highly potent esters of estradiol in males (Fig. 3). It may appear also in females (Fig. 4).

Frequently a tumoral seed can be seen also on the surface of the left kidney and not rarely on the right one. Almost constant phenomena are small nodules on the albuginea of the testicle (Figs. 5 and 6).

Fibrous strands induced in the peritoneum by a prolonged treatment with estrogens (22) almost never failed to appear in our animals (Table II). They were more pronounced in the 320 and 160 μgm. groups than in the 80 and 40 μgm. groups; but in the latter groups the fibrous strands also were sometimes very conspicuous (Fig. 2).

Table I: Extragenital Tumors in 37 Male and 23 Female Guinea Pigs; 36 to 39 Injections of Estrogens in Three Months

Sex of guinea pigs	Stilbestrol per injection μgm.	Number injected	Number of animals with extragenital tumors, class 1 or larger	Hexestrol per injection μgm.	Number injected	Number of animals with extragenital tumors, class 1 * or larger
Females	80	8	5	40	10	5
Females	..	..	..	80	5	4
Males	80	9	0	40	10	0
Males	320	9	1	160	9	1

* Class 1 tumors are not less than 1 mm. in diameter.

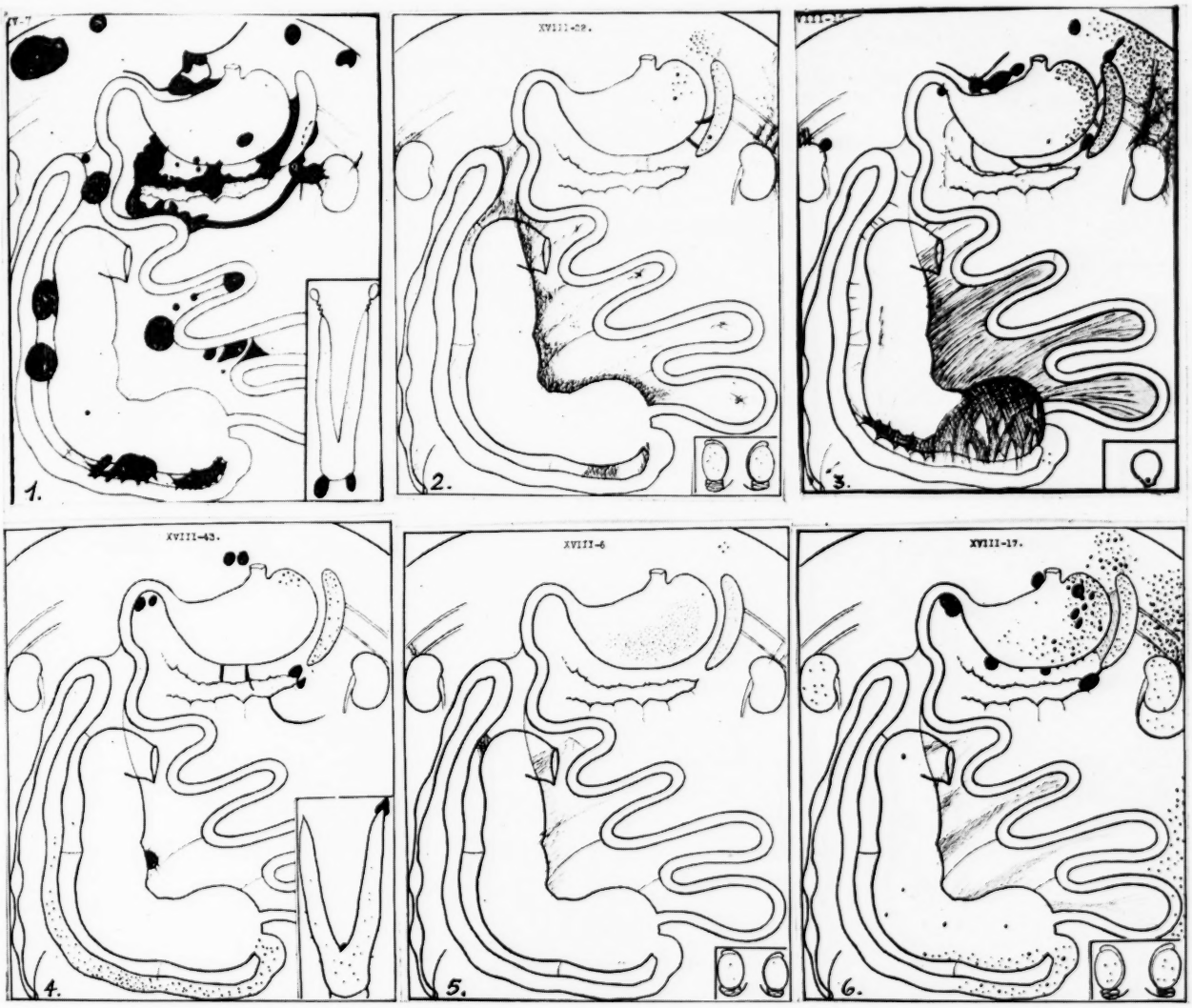
Table II: Conjunctive Tumoral Reactions of 37 Male Guinea Pigs; 36 to 38 Injections of Estrogens in Three Months

	μgm. per injection	Number of animals	Number of animals with tumoral seed on the: Testicle	Stomach	Intestine	Kidney: Left	Kidney: Right	Abdominal wall: Left	Abdominal wall: Right	Spleen	Number of animals with fibrous strands
Stilbestrol	80	9	9	5	0	2	2	5	0	3	9
Stilbestrol	320	9	9	6	1 *	6	2	9	0	8	9
Hexestrol	40	10	7	7	0	4	2	9	0	7	9
Hexestrol	160	9	9	6	0	1	0	7	0	6	9

* See Fig. 6.

Discussion

The results of our work with prolonged administration of stilbestrol and hexestrol in guinea pigs leave no doubt as to the fact that with artificial estrogens conjunctive proliferation is different in females and males. There is the same sex difference as formerly



Extragenital fibroids and tumoral seeds are indicated in solid black

FIG. 1.—Noncastrated female guinea pig—36 injections of 80 μgm. of estradiol given as 17-benzoate in the course of 83 days (IV, 7). Typical female reaction. Numerous extragenital subserous tumors on the abdominal wall, diaphragm, stomach, omentum, pancreas, spleen, and liver. Tumors on the dorsal surface of the uterus.

FIG. 2.—Noncastrated male guinea pig—36 injections of 40 μgm. of hexestrol in the course of 83 days (XVIII, 22). Typical male reaction. This may be observed also in the female with small doses and very exceptionally with large ones. Tumoral seed on the spleen, stomach, abdominal wall, and diaphragm on the left. Adhesions between spleen and stomach. Fibrosis around kidneys, mesentery, and mesocolon highly pronounced. Small nodules on the tunica albuginea testis.

FIG. 3.—Castrated male guinea pig—44 injections of 80 μgm. of estradiol given as 17-caprylate in the course of 104 days (XVIII, 15). Exceptional type of male reaction combined with tumors as observed only with large doses of potent esters and during long treatment. Highly pronounced tumoral seed of typical left-sided localization. Fibrous strands of the mesentery and kidney. Fibrous cover on the cecum. Numerous subserous tumors on the smaller curvature of the stomach, pylorus, omentum, liver, right kidney, diaphragm, upper and under pole of the spleen. In the lower right: small nodule on the urinary bladder.

FIG. 4.—Castrated female guinea pig—38 injections of 80 μgm. of stilbestrol in the course of 87 days (XVIII, 43). Typical female reaction, but combined with conspicuous tumoral seeds. Uterus (7.7 gm.) with numerous small subserous nodules and larger nodule between the horns. Apical tumor on the left horn. Several tumors in the omentum, in the mesentery, near the pancreas. Fibrous strands in the omentum. Tumoral seed on the spleen, stomach, and ascending colon.

FIG. 5.—Noncastrated male guinea pig—38 injections of 80 μgm. of stilbestrol in the course of 87 days (XVIII, 6). Typical male reaction. Tumoral seed on the spleen, stomach, and abdominal wall on the left. Fibrous strands in the mesentery and on the upper part of the kidney. Small nodules on the tunica albuginea testis.

FIG. 6.—Male guinea pig—38 injections of 320 μgm. of stilbestrol in the course of 88 days (XVIII, 17), the only animal out of 18 which showed tumors. Exceptional type of male reaction—combination with tumors. Tumoral seed on the spleen, stomach, abdominal wall, diaphragm, and kidney extending to the right as seen especially on the stomach, and to the cecum and ascending colon. Fibrous patches, partly pedunculated, on the surface of the stomach. Cystic condition of the left kidney; nodules on the surface of the cyst which belongs entirely to the capsule of the kidney. Adhesions between spleen and stomach. Fibrous strands in the mesentery. Multiple tumors near the stomach and duodenum and between the spleen and pancreas. Small nodules on the tunica albuginea testis.

observed with natural estrogens. The tumoral seed especially on the spleen and the surrounding parts of the abdominal cavity and fibrous strands in the mesentery and mesocolon exhibits the typical pattern elicited in the male. Indeed we must emphasize, as we have already insisted above, that a similar reaction may also be seen in the female, especially with a small dosage of estrogens. On the other hand, the individual spherical tumors of a certain size typical of the female may be seen also in the male, especially after large dosage of estrogens. The difference in reaction between females and males, however, was as pronounced with artificial estrogens as with the natural follicular hormones. This is shown by comparison of Figs. 2 and 5 with Figs. 1 and 4.

Since the metabolic fate of artificial estrogens is not identical with that of the natural follicular hormones our findings suggest that the sex difference in the conjunctive tumoral reaction towards estrogens, natural or artificial, does not depend upon a sex difference in the metabolism of estrogens. There must be some sex-linked factor which determines a sex difference in the susceptibility of the tissues for the proliferative action of estrogens and one may tentatively suggest that this factor is also of endocrine origin, probably deriving from the anterior hypophysis.

The difference in the tumoral reaction of females and males as described by our group can be interpreted as a quantitative one. The male type of reaction (tumoral seed on the left side of the body) is that of a female with an exceptionally low susceptibility; with larger doses tumors may appear also in the male (Figs. 3 and 6). Though all of this is certainly in favor of a quantitative interpretation the following fact is somewhat contrary to our purely quantitative conception: in rare individuals, spherical tumors appear also in the male (Fig. 6), while the male pattern of reaction is never extinguished. The individual spherical tumors are so to say superimposed on the male pattern of reaction. Another feature, however, is again in favor of the quantitative interpretation: together with individual tumors appearing in the male the left-sided tumoral seed may extend to the right as a consequence of injection of larger doses (6, 7). There is evidently a graded reactivity of the left and right side of the body towards estrogens. This graded reactivity of left and right belongs also to the female as has been shown by our observations in hundreds of experiments (9, 10, 22). We are as yet unable to explain this remarkable phenomenon. The spleen is a most important starting point of conjunctive peritoneal tumorigenesis elicited by estrogens. Whether this means that the spleen has something to do with the intimate dynamics of this type of tumorigenesis is indeed as yet not certain (20, 21).

Summary

A comparative study of the conjunctive tumoral reaction towards prolonged treatment with artificial estrogens (stilbestrol, hexestrol) has been made in female and male guinea pigs.

As with follicular hormones there was a conspicuous sex difference in the reactive behavior toward artificial estrogens.

Quantities of stilbestrol or hexestrol sufficient to elicit in the female a high frequency of large spherical extragenital peritoneal fibroids, induce in the male only a tumoral seed on the spleen, the stomach, and the surrounding left-sided parts of the peritoneum, and peritoneal fibrous strands.

With quantities four times greater, the tumoral seed and the fibrous strands become more conspicuous and only very exceptionally may individual spherical tumors appear in the male in contrast to those observed in the female.

This gives evidence that in respect to the sex difference in the conjunctive tumoral reaction towards estrogens natural follicular hormones and artificial estrogens behave in a similar way.

Since the metabolic conditions of natural and artificial estrogens in the body are dissimilar, our findings suggest that the sex difference in the tumoral reaction is not due to metabolism of hormones differing according to sex but to some other sex-linked factor which determines a different susceptibility of the reacting tissue. It is tentatively suggested that this factor may be hormonal, probably originating in the anterior hypophysis.

The fact that the male pattern of reaction is observed also in the female with a lower dosage of the estrogen, and the female pattern is observed in the male with a higher dosage, is in favor of a quantitative interpretation of the described sex difference in the tumoral reaction.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

DAVIS, J. H., K. M. MURPHY, and L. A. EMGE. [Stanford Univ. Sch. of Med., San Francisco, Calif.] EFFECT OF DIBENZANTHRACENE ON TRANSPLANTABLE MAMMARY ADENOFIBROMA OF THE WHITE RAT. Am. J. Path., 17: 93-101. 1941.

Dibenzanthracene regularly produced sarcomas in rats at the site of injection after an interval of at least 100 days. These tumors were readily transplantable and somewhat more malignant than the sarcomas derived from the adenofibromas of the breast. Dibenzanthracene did not induce malignant change in a transplantable mammary adenofibroma but inhibited the rate of its growth in female hosts.—H. B.

FALIN, L. I., and K. E. GROMZEWA. [Histol. Lab. des Med. und Stomatol. Inst., Smolensk, U.S.S.R.] ZUR PATHOGENSE DER EXPERIMENTELLEN TERATOIDEN GESCHWÜLSTE DER GESCHLECHTSDRÜSEN. II. MITTEILUNG. TERATOIDE GESCHWÜLSTE DER GESCHLECHTSDRÜSEN BEI HÄHNEN, ERZEUGT DURCH INJEKTIONEN VON $Zn(NO_3)_2$—LÖSUNG. [PATHOGENESIS OF EXPERIMENTAL TERATOID TUMORS OF THE SEX ORGANS. II. TERATOID TUMORS OF THE SEX ORGANS OF THE COCK PRODUCED BY INJECTIONS OF ZINC NITRATE SOLUTION.] Virchows Arch. f. path. Anat., 306:578-588. 1940.

As has been previously demonstrated for other zinc salts (chloride, sulfate) the authors succeeded in producing comparable teratoid tumors in the testicle of the cock by intratesticular injection of zinc nitrate. Of 39 animals receiving 0.1 to 0.2 cc. of a 10% solution of zinc nitrate and sacrificed between 6 and 100 days, 4 had small tumors after 41, 62, 84, and 85 days, respectively.—M. J. E.

JONES, R. N. [Harvard Univ., Cambridge, Mass.] FURTHER OBSERVATIONS ON THE ABSORPTION SPECTRA OF DERIVATIVES OF 1,2-BENZANTHRACENE. J. Am. Chem. Soc., 63:151-155. 1941.

In an earlier paper (J. Am. Chem. Soc., **62**:148-152. 1940), the author recorded data on the ultraviolet absorption spectra of a number of alkyl substituted 1,2-benzanthracenes. Probably as a result of common structural influences, there is some correlation between carcinogenic activity of the compounds and the shift of the absorption curves towards longer wave lengths (bathochromic shift); the magnitude of the shift is greatest for derivatives having alkyl substituents at the reactive meso positions of the 1,2-benzanthracene molecule. The intensities of the maxima and the shapes of the curves are essentially similar to those of 1,2-benzanthracene. In the present article, it is noted that amino, hydroxy, and methoxy groups produce considerable alteration in the spectrum whereas the isocyanate, nitro, and carboxylic acid groups have less effect. Qualitative observations on the fluorescence of many of the compounds are included.—H. J. C.

LAW, L. W. [Jackson Memorial Lab., Bar Harbor, Me.] THE INDUCTION OF LEUKEMIA IN MICE FOLLOWING PERCUTANEOUS APPLICATION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE. Cancer Research, 1:564-571. 1941.

Three types of neoplastic reactions of the blood-forming organs have been observed in mice of the Dba strain following percutaneous application of a 0.5% solution of 9,10-dimethyl-1,2-benzanthracene. A general lympomatosis (lymphoid leukemia) occurred in 101 of 119 mice (84.9%). The mean latent period was 107.6 days. The earliest lesion appeared 45 days following initial painting. Myeloid leukemia appeared in 8 mice (6.7%) and monocytic leukemia in 2 mice (1.7%).

The optimal dosage of carcinogen required for induction of leukemia following painting was 0.5%. Subcutaneous injections of subtoxic doses were less effective.

The factors of sex, subline, or foreign uterine stimulus did not affect the incidence of induced leukoses. However, a significant decrease in the incidence of induced lymphoid leukemia resulted in mice of the Dba strain which were from 2+ to 16 months of age at the beginning of painting. Only 7 cases (15.5%) occurred. The chief reaction induced in old mice was a benign extramedullary myelopoiesis with associated neutrophilia.

In month-old MacDowell-Bagg C strain mice, following similar treatment with 9,10-dimethyl-1,2-benzanthracene, lymphoid lesions resulted in only 5 of 44 mice (7.5%). Three of these lesions were of the generalized lymphomatosis type and 2 were mediastinal lymphosarcomas. The majority of the reactions involved the myeloid elements of the blood-forming organs. Five cases of myeloid leukemia and 28 cases of benign extramedullary myelopoiesis were observed.

A condition of extramedullary myelopoiesis which appeared coincident with and was transmissible by an induced epithelioma was observed.

Other inbred strains of mice painted with a 0.5% solution of 9,10-dimethyl-1,2-benzanthracene were the leaden, C_2H, Swiss, ABC, N, C57 black, and C57 brown. Only one, the Swiss strain, reacted in the formation of generalized lymphomatosis.—Author's abstract.

MENKIN, V. [Harvard Univ. Med. Sch., Boston, Mass.] CELLULAR INJURY IN RELATION TO PROLIFERATIVE AND NEOPLASTIC RESPONSE. Cancer Research, 1:548-556. 1941.

An inflammatory exudate is considered as a fluid containing products of cellular injury. With this in view, normal cells have been exposed for prolonged intervals to the action of exudative material and its ultimate effect has been studied.

Frequent injections of an exudate into the subcutis of a rabbit's ear over a protracted period of time induce a severe inflammatory reaction. Cessation of these injections is followed months later by sustained and marked poliferative activity. This is characterized by atypical epithelial changes as evidenced by hyperplasia and metaplasia of the normal epithelial layer. Foci of keratinization also form a conspicuous feature of the reaction.

Furthermore, the cartilage of the ear, at the site of injections, manifests marked proliferative activity giving rise to small, palpable, and indurated structures. Microscopically the cartilage frequently appears as irregular unevenly shaped areas of proliferative cells derived from the perichondrium. Both fibrous and hyaline cartilaginous cells form the essential elements of the proliferative reaction. Areas of metaplastic ossification with hematopoietic centers are, however, also in evidence in such areas. In several instances the marked proliferative activity on the part of the epithelium and cartilage has been found to be associated with papillomatous tufts and with morphological structures consistent with chondroma.

Serum injections or the repeated administration of croton oil in olive oil emulsified with serum have failed to induce any marked proliferative responses following acute inflammation.

The available evidences suggest the presence of one or possibly several proliferation-producing factors liberated by injured cells into inflammatory exudates to explain the foregoing observations. These studies are being continued further in an endeavor to elucidate the nature of such growth-stimulating substances.—Author's abstract.

NAGAO, N. [Sasaki-Lab., Tokyo] EXPERIMENTELLE ERZEUGUNG DES SARKOMS BEI WEISSEN RATTEN DURCH KOMBINIERTE SUBKUTANE APPLIKATION VON KIESELGUR UND o-AMINOAZOTOLUOL. [EXPERIMENTAL PRODUCTION OF SARCOMAS IN WHITE RATS BY THE COMBINED SUBCUTANEOUS APPLICATION OF KIESELGUHR AND o-AMINOAZOTOLUENE.] Gann, 35:1-6. 1941.

Olive oil suspensions of kieselguhr (10%) with and without o-aminoazotoluene (1%) were injected subcutaneously into groups of white rats as follows: I. 30 rats received weekly injections (at the same spot) of 0.5-1.0 cc. of kieselguhr suspension containing 1% o-aminoazotoluene. The diet consisted of unpolished rice and greens. II. 20 rats received injections of kieselguhr suspension without o-aminoazotoluene. The polished rice diet contained 0.1% o-aminoazotoluene. III. 10 rats received injections as in II and diet as in I. Some rats in this group also received injections of suspensions of ivory as controls.

No tumors were found in group III. In group I, 4 sarcomas were found after 330, 390, 398, and 453 days. In group II, 2 sarcomas developed after 476 and 648 days. All sarcomas were formed at the injection sites. Transplantation was attempted with only 1 sarcoma which was successful through 3 generations. The tumors were spindle celled or polymorpho-celled sarcomas and contained neither multinuclear foreign body giant cells nor kieselguhr residues. According to the author these results prove that o-aminoazotoluene, in addition to causing hepatomas and cholangiomas, can, under certain circumstances, promote sarcoma formation.—P. P. C.

NAGAO, N. [Sasaki-Lab., Tokyo] ÜBER DIE FÜTTERUNGSVERSUCHE MIT 4'-METHYL-4-N-DIMETHYLAMINOAZOBENZENE UND WEITEREN POLYMETHYLDERIVATEN BEI WEISSEN RATTEN. [FEEDING EXPERIMENTS WITH 4'-METHYL-4-N-DIMETHYLAMINOAZOBENZENE AND OTHER POLYMETHYL DERIVATIVES WITH WHITE RATS.] Gann, 35:8-20. 1941.

The carcinogenic activity of the compounds, 4'-methyl-4-N-dimethylaminoazobenzene(I), 2,4'-dimethyl-4-N-dimethylaminoazobenzene(II), and 3,4'-dimethyl-4-N-dimethylaminoazobenzene(III) was studied. The compounds were dissolved in olive oil and mixed with unpolished rice to give concentrations of 0.03-0.2%. One group of 31 rats received the diet containing I. Fourteen of these survived for longer than 210 days, 11 of which developed hepatic carcinomas (3 with metastases) between 210 and 402 days. A second group of 33 rats received the diet containing II, 14 of which survived for more than 240 days. Of these 7 developed benign cysts, one of which showed an adjacent area of malignancy. No other tumors were observed after 610 days. A third group of rats received the diet with III. No malignant changes were observed after 610 days. The author concludes as follows: 1. The introduction of a methyl group in the para(4') position of 4-N-dimethylaminoazobenzene does not prevent carcinogenesis but results in a slight inhibition. The resulting tumors are chiefly cholangiomas or of the mixed type. 2. The introduction of a second methyl group results in a stronger inhibition of carcinogenesis, with the result that only benign cystic cholangiomas are produced. 3. The introduction of 2 methyl groups in positions 3 and 4' causes a still greater inhibition. To date compound III has produced neither cysts nor carcinomas in liver. Gross and histologic descriptions of the livers from the 3 groups are presented in detail.—P. P. C.

ROFFO, A. H. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires] ALQUITRÁN DE TÉ CANCERÍGENO. [CARCINOGENIC TAR FROM TEA.] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 55:661-698. 1940.

Dried extract of leaves of the upper part of the tea plant contain 16.5% fat. The author found 2 gm. of lipoids in 1 kg. of tea. By distillation at slowly increased temperature reaching 350° after 3 hours a tar was extracted from tea, and some of its physical characteristics and its carcinogenic power were studied. Spectrographic and fluorescent constants were found analogous to those of tar from tobacco and coffee. Of 11 rabbits whose ears were painted every other day with the tar, 9 developed cancer, 1 papillomas, and 1 proved to be resistant. Lesions were first noticeable from 46 to 175 days after the treatment was started. The author thinks that these findings have only a theoretical interest since the carcinogenic hydrocarbons are not soluble in water, and therefore are not found in the usual infusion of tea. The text contains 23 illustrations.—M. D-R.

Hormones

CLEVELAND, R., D. PHELPS, and J. C. BURCH. [Vanderbilt Univ. Med. Sch., Nashville, Tenn.] EXPERIMENTAL PRODUCTION OF GLANDULAR CYSTIC HYPERPLASIA IN CASTRATED MACAQUES. Endocrinology, 28:659-663. 1941.

Glandular cystic hyperplasia was produced in 1 of 3 castrated macaques receiving 2,500 r.u. estradiol benzoate daily for a period of 3 months, and in 2 of 3 castrated macaques receiving 5,000 r.u. daily for the same period of time. Cystic dilation of the glands occurred only in animals which had been subjected to endometrial biopsy by laparotomy 4 to 6 times previous to the start of injections. This led to the suggestion that the ratio of estrogenic material to the amount of endometrial tissue was an important factor in producing the hyperplasia.—C. A. P.

GARDNER, W. U., and T. L. CHAMBERLIN. [Yale Univ. Sch. of Med., New Haven, Conn.] **LOCAL ACTION OF ESTRONE ON MAMMARY GLANDS OF MICE.** Yale J. Biol. & Med., 13:461-465. 1941.

Growth of mammary tissue occurred in male hybrid mice at the site of application to the skin of estrone in amounts of 0.02 to 0.16 μgm. in alcohol daily. In 4 of 21 animals after 15 days, the effect was local, in 7 the growth was greater at the site of application than elsewhere, and in 9 generalized stimulation of the mammary tissue was observed. Only one animal showed no effect. This direct local action of estrone occurred despite the fact that the rudimentary glands in these mice were located in the subcutaneous tissue and were not attached to the skin.—A. A. L.

LIPSCHÜTZ, A., F. RODRÍGUEZ, and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] **CONTINUOUS AND DISCONTINUOUS TREATMENT WITH ESTROGENS IN EXPERIMENTAL TUMORIGENESIS.** Endocrinology 28:664-668. 1941.

Of 6 castrated female guinea pigs receiving 20γ of estradiol benzoate twice weekly, all developed uterine tumors. Three of these had palpable tumors by the 113th day of treatment (total dose of 940γ). The other 3 were continued for 155 to 312 days. One of these developed a large apical tumor and the other 2 subserous fibroids. A 7th animal which received 80γ of estradiol benzoate 3 times weekly was found at autopsy, at 79 days, to have uterine and extra-uterine tumors. All of these uteri contained many cystic glands which sometimes extended into the vagina. In some cases the hypertrophic mucosa showed adenomatous changes. Repeated uterine bleeding occurred after 2 to 4 months.

The same experiment was repeated with the modification that rest periods of from 15 to 19 days followed each week of injection (3 injections of 20γ or 80γ per week). This procedure was continued for a year without the occurrence of uterine bleeding or the formation of uterine or extra-uterine fibroids. Only slight hyperplasia of the uterine endometrium occurred. However, some glandular cysts were found at the cervical junction and in 2 cases epithelial cords had penetrated the submucosa of the vaginal fornix. The authors concluded that the toxic effects of estrogens may be prevented in the guinea pig by allowing sufficiently long rest intervals during the course of an interrupted series of estrogen injections.—C. A. P.

LIPSCHÜTZ, A., and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] **PREVENTION OF EXPERIMENTAL UTERINE AND EXTRAUTERINE FIBROID BY TESTOSTERONE AND PROGESTERONE.** Endocrinology, 28:669-675. 1941.

In castrated female guinea pigs uterine fibroids induced by estrogen injections were completely prevented by the simultaneous injection of 50 times as much testosterone as estrogen or 150 times as much progesterone as estrogen. With pellet implantation the ratio of estrogen to progesterone required was only 1:13. The same doses also gave about 90% protection against extra-uterine (visceral) tumoral reactions. The histology of the tumors of the uterine endometrium was not given.—C. A. P.

LIPSCHÜTZ, A., L. VARGAS, JR., and J. PALMA. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] **SEX DIFFERENCE IN THE CONJUNCTIVE TUMORAL REACTION OF THE GUINEA PIG TOWARDS NATURAL AND ARTIFICIAL ESTROGENS.** Cancer Research, 1:575-579. 1941.

The conjunctive proliferative reaction following prolonged treatment with artificial estrogens (stilbestrol, hexestrol) has been studied in female and male guinea pigs. As with natural follicular hormones there was a conspicuous sex difference in the reactive behavior with artificial estrogens. In the male there was only the so-called "tumoral seed" on the spleen, the stomach, and the surrounding parts of the peritoneum on the left side, and there were peritoneal fibrous strands. Individual spherical peritoneal fibroids common in the female subjected to a similar treatment appeared only rarely in the male even when the quantity of the administered hormone was several times greater than that given to the female. It is suggested that the sex difference in the tumoral reaction is due to some sex-linked factor which determines a sex different susceptibility of the reacting tissue. The male pattern of reaction is observed also in the female with a lower dosage, and the female pattern is observed in the male with a higher dosage. This points toward a quantitative interpretation of the described sex difference.—Authors' abstract.

MARK, J., and G. R. BISKIND. [The Johns Hopkins Univ., Baltimore, Md.] **THE EFFECT OF LONG TERM STIMULATION OF MALE AND FEMALE RATS WITH ESTRONE, ESTRADIOL BENZOATE AND TESTOSTERONE PROPIONATE ADMINISTERED IN PELLET FORM.** Endocrinology, 28:465-477. 1941.

Estrone and estradiol benzoate pellets (average daily absorption of 7.7γ and 8.5γ respectively) when remaining in normal or castrated females for long periods of time produced all the atypical growth changes in the genital tract (metaplasia, cystic dilation of uterine glands, marked hyalinization and fibrosis of the myometrium, and marked keratinization of the uterus) which follow injections of the same hormone for a similar period of time. Pyometra caused death as early as the 66th day. Precancerous breast changes occurred in one rat after 115 days. A definite adenocarcinoma was found in the breast of another after 223 days. Adrenals and pituitaries were markedly enlarged. In one of the treated castrated females the weight of the hypophysis reached 40 mgm. due to chromophobe hyperplasia. Testosterone propionate pellets (average daily absorption of 59γ) caused reduction of weight of the genital tract, adrenals, and hypophysis. When pellets of both estrogens and androgens were given simultaneously the one did not affect the absorption of the other, nor did the testosterone propionate inhibit the actions of the estrogens. Organ weights were taken on the above animals at the end of the period of treatment and also on normal and castrated males treated with testosterone propionate. The rate of absorption of the pellets was not influenced by the site of introduction (whether into subcutaneous tissue, spleen, liver, or kidney) or by either the physiological need of the animal or the simultaneous introduction of more than one pellet.—C. A. P.

WOLFE, J. M., E. BURACK, and A. W. WRIGHT. [Albany Med. Coll., Albany, N. Y.] **ABNORMAL GROWTH IN A STRAIN OF RATS CHARACTERIZED BY LOW FERTILITY**

AND A HIGH INCIDENCE OF BENIGN MAMMARY TUMORS. Endocrinology, 27:883-887. 1940.

The Albany (A-S) strain of rats is characterized by spontaneous reproductive disorders and a relatively high incidence of benign mammary tumors in the older females. The weaning weights, growth rates, and final weights of both males and females of this strain were found to be decidedly inferior to those of a control V-S (Vanderbilt) strain in which fertility is excellent and the incidence of mammary tumors comparatively low. At 165 days the growth rates of sterile (A-S) females were statistically inferior to those of nonsterile virgins of the same strain. In both the A-S and the V-S strain breeding females grew more rapidly and attained a greater weight than did nonbreeding females. It is suggested that this difference is due to a suppression of growth by estrogen which in the breeding female is counteracted by progesterone. The slower growth rate and lower attained size of the A-S strain as compared to the V-S may be due to a spontaneous pituitary disfunction.—C. A. P.

WRIGHT, A. W., G. H. KLINCK, JR., and J. M. WOLFE. [Albany Med. Coll., Albany, N. Y.] THE PATHOLOGY AND PATHOGENESIS OF MAMMARY TUMORS OCCURRING SPONTANEOUSLY IN THE ALBANY STRAIN OF RATS. Am. J. Path., 16:817-834. 1940.

Fibro-epithelial mammary tumors have occurred with increasing frequency in the A-S strain of rats over a period of several years. Certain endocrine disorders have also been observed during this interval: changes in the sex cycle of the female, diminished fertility in both males and females, prolonged gestation and spontaneous fetal absorption, and deficient growth.

The tumors occurred in breast tissue in the mammary line; the size varied, but at times they weighed as much as the host. The tumors were encapsulated fibro-epithelial growths (adenofibromas) or adenomas, fibromas, or cystadenomas; one was a carcinoma. The earliest stage in the development of these tumors was thought to be represented by zones of increased interstitial fibrous tissue which involved one lobe or surrounded groups of small ductules and acini. Analysis of the tumors failed to reveal estrin and prolonged administration of estrin produced only proliferation of the glandular tissue.—H. B.

Viruses

BARRETT, M. K. [Rockefeller Inst. for Med. Research, New York, N. Y.] THE ANTIGENIC NATURE OF PURIFIED CHICKEN TUMOR AGENT. Cancer Research, 1:543-544. 1941.

When products of the highest degree of purity presently obtainable are used, there is a demonstrable immunologic difference between the extracts of chicken tumor I and similar material from normal tissues.—Author's summary.

MILLER, G. L., and W. M. STANLEY. [Rockefeller Inst. for Med. Research, Princeton, N. J.] ACETYL AND PHENYLUREIDO DERIVATIVES OF TOBACCO MOSAIC VIRUS. Science, 93:428-429. 1941.

A large part of the free amino groups present in tobacco mosaic virus protein (up to 3,000 NH_2 groups per molecule) could be covered by acetylation with ketene or by treatment with phenyl isocyanate without a decrease in virus activity. In contrast to the findings of Schramm and Müller (Ztschr. f. physiol. Chem., **266**:43-55. 1940.), complete coverage of the amino groups entailed some loss in biological potency. Acetylation of 75 to 83% of the amino groups led to 25 to 50% inactivation. Acetylation of 70% of the NH_2 groups and 20% of the tyrosine plus tryptophane residues yielded a derivative of unchanged biological activity. The pathological symptoms produced in plants infected with the acetyl and phenylureido derivatives were undistinguishable from those caused by the original virus strain. Phenylureido virus preparations in which 43 to 63% of the amino groups were covered showed no significant change in specific virus activity when compared with ordinary virus. The homogeneity of the materials, as determined in the analytical ultracentrifuge, was not measurably altered by the 2 chemical reagents employed. However, when examined in the electrophoresis apparatus of Tiselius at pH 7.3, the acetyl and phenylureido derivatives each showed mobilities close to -9.3×10^{-5} cm.^{2}/volt sec. as compared with -8.3×10^{-5} cm.^{2}/volt sec. for the normal virus. The 2 derivatives were very homogeneous in electrochemical respect and when mixed with unaltered virus could be readily separated from the latter by electrophoresis. The results are stated to demonstrate that a large portion of certain functional groups of the virus molecule may be altered without interfering with the basic reaction of virus reproduction. It is important to note that the virus prepared from plants inoculated with the acetyl or phenylureido derivatives possessed a normal amino nitrogen content, demonstrating that "infecting virus molecules may not necessarily function as exact patterns for reproduction."—K. G. S.

SCHRAMM, G., and H. MÜLLER. [Kaiser-Wilhelm-Inst. f. Biochem., Berlin-Dahlem, Germany] ZUR CHEMIE DES TABAKMOSAIKVIRUS. UEBER DIE EINWIRKUNG VON KETEN UND PHENYLISOCYANAT AUF DAS VIRUSPROTEIN. [CHEMISTRY OF TOBACCO MOSAIC VIRUS. EFFECT OF KETENE AND PHENYLISOCYANATE ON THE VIRUS PROTEIN.] Ztschr. f. Physiol. Chem., 266:43-55. 1940.

The virus protein used in these experiments was isolated in crystalline form by fractional precipitation with ammonium sulfate from the press juice from frozen tobacco plants, harvested 4 weeks after infection with the original tobacco mosaic virus strain of Stanley. When examined in 0.2 M salt solution of pH 8 in an air driven analytical ultracentrifuge, employing Toepler's schlieren band method, only one homogeneous component of a sedimentation constant, $s_{20}=180$ was observed in freshly prepared solutions. Upon ageing, a second component with $s_{20}=209$ appeared which was regarded as the dimer of the lighter particle. Assuming an axis ratio of 1:10 for the monomer and of 1:20 for the dimer, which is supported by electron optical studies of H. Trurnit in M. v. Ardenne's laboratory, molecular weights of 23×10^6 and 46×10^6 respectively for the 2 forms are calculated. The relationship between the 2 forms is that of a reversible equilibrium system. At pH 8.2 the equilibrium is in favor of the monomer, at pH 5.8 it is greatly in favor of the dimer and even larger aggregates tending to form paracrystalline needles. In the electrophoresis apparatus of Tiselius the material appeared electrochemically homogeneous under all experimental conditions. The

amino nitrogen content, as determined according to van Slyke, was 0.25%, corresponding to 0.5% lysine in the material. Upon treatment with ketene in 1.0 M acetate buffer of pH 5.8 for 10 minutes all free amino groups are acetylated, yielding a *fully active* virus derivative, crystallizing in the form of paracrystalline needles like the starting material and exhibiting the same behavior in the analytical ultracentrifuge. The acetyl derivative is electrochemically homogeneous and shows a higher anodic mobility than the original virus protein. A mixture of acetylated and nonacetylated virus may be readily resolved by electrophoresis. The acetylation of the phenolic hydroxyl groups in the molecule upon prolonged ketene treatment leads to an increasing loss of biological potency without interfering with the crystallizing ability of the material. The phenylcarbamino derivative obtained by treating the virus with phenylisocyanate at pH 8 and 0° C. is fully active and may be obtained in the form of crystalline needles at pH 4.6. The sedimentation constant, determined at pH 8, is the same as that of the starting material. It follows that esterification of the free amino groups of the virus protein does not impair its biological activity. The results obtained with ketene, in contrast to those obtained with phenylisocyanate, are analogous to observations made previously on pepsin and insulin by Herriott and by Stern and White respectively.—K. G. S.

SEALY, W. C. [Duke Univ. Sch. of Med., Durham, N. C.] THE INDUCTION OF LIVER NECROSIS IN RABBITS BY THE COMBINATION OF EXPERIMENTAL HYPERTHYROIDISM AND SHOPE PAPILLOMA. Ann. Surg., 113:572-578. 1941.

In 12 of 15 domestic animals with sloughing Shope papillomas, experimental hyperthyroidism, induced by feeding desiccated thyroid, resulted in widespread central necrosis. None of the two control series showed this change. The authors suggest that the responsible factors are the tumor necrosis and infection rather than the virus. A bibliography of 22 authors is given.—A. M.

Genetics

STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] A GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE. II. THE INFLUENCE OF SPINDLE CELL SARCOMA AND OF CARCINOMA OF THE SKIN UPON EACH OTHER. Cancer Research, 1:572-574. 1941.

Carcinoma of the skin is the earliest type of tumor induced in NH mice by the injection of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil and injected subcutaneously at 60 days of life. The latent period for the induction of spindle cell sarcoma by the same technic is longer than it is for carcinoma of the skin. Both carcinoma of the skin and spindle cell sarcoma frequently occur together in the same tumor mass and when they do the latent period and the frequency distribution of either type is not disturbed by the presence of the other type. The origins of carcinoma of the skin and of spindle cell sarcoma are apparently independent of each other and are probably brought about by a peculiar biological constitution of the individual which gave rise to them.—Author's summary.

Biochemistry and Nutrition—Chemotherapy

ALLEN, F. M. [New York, N. Y.] COMBINATIONS OF LEAD, ARSENIC, AND OTHER CHEMICALS WITH EXPERIMENTAL ASPHYXIA OF TUMORS. J. Lab. & Clin. Med., 26:1120-1128. 1941.

The attempts to use local ligations to obtain an intensified tumor-killing effect of the drugs was unsuccessful, as was the effort to use the drugs in local or systemic application to augment the effect of asphyxia. In conformity with previous work indicating that the tumor-killing action of local ligation is due neither to the direct asphyxia nor to the excess oxygenation of the ensuing hyperemia, the combination of ligation with various agents serving to increase or to depress cell respiration proved negative. Cures were obtained in some mouse tumors and also in favorably situated rat tumors with arsenic.—H. G. W.

ANDO, T. [Path. Inst. der Kais. Univ. zu Kyoto] EINFLUSS DER FUTTERMITTEL AUF DIE EXPERIMENTELLE LEBERKARZINOMENTSTEHUNG (III MITTEILUNG). EXPERIMENTELLE LEBERKARZINOMENTSTEHUNG UND GETREIDE. (ZEITDAUER DER ZUGABE DES KANZEROGENEN MITTELS: 180 TAGE.) [EXPERIMENTAL CARCINOMA OF THE LIVER AND DIET; PERIOD OF FEEDING CARCINOGEN, 180 DAYS.] Gann, 35:62-63. 1941.

Two groups of 50 rats each were fed o-aminoazotoluene mixed with wheat (group I) and unpolished rice (group II) for 180 days. The rats were then fed wheat alone or unpolished rice alone until the death of the animals. In group I, 3 liver carcinomas were found after 561, 705, and 756 days. In addition 3 mammary tumors were noted after 717, 750, and 756 days. In group II, 4 liver carcinomas were observed after 480, 649, 672, and 695 days. On the basis that only 3 of the 5 rats in group I which survived 700 days showed liver carcinomas, while all of the rats in group II which survived 600 days showed liver carcinomas, the author concludes that wheat feeding exerts a prophylactic effect of hepatic carcinogenesis by o-aminoazotoluene. No details of the mammary tumors are given other than that one of these grew to have a diameter of 8 cm.—P. P. C.

BAYERLE, H. [Path. Inst. der Univ. München, Germany] DIE GESCHWULSTTHEORIE NACH F. KÖGL. [KÖGL'S TUMOR THEORY.] Monatschr. f. Krebsbekämpf., 9:1-11. 1941.

The author reviews the present status of knowledge regarding the occurrence of the d(-) form of glutamic acid in tumor proteins, as first described by Kögl and Erxleben. He is of the opinion that abnormal amino acids, although demonstrable in neoplastic tissue, bear no specific relationship to the etiology of malignant disease. Bayerle found that somewhat smaller quantities of partially racemized glutamic acid than originally obtained by Kögl and Erxleben were extractable from malignant neoplasms. However, noncarcinomatous tissue was found to give a higher yield of d(-)glutamic acid than reported by the original investigators as present in normal organs. The chemical procedures of Kögl were followed, but quantitative data are not included.—M. J. E.

BERGER, J., M. J. JOHNSON, and C. A. BAUMANN. [Coll. of Agric., Univ. of Wisconsin, Madison, Wis.] EN-

ZYMATIC HYDROLYSIS OF d-PEPTIDES. J. Biol. Chem., 137:389-395. 1941.

The authors were unable to confirm the reported occurrence of d-peptidases in the blood serum of humans with cancer. It was found that serums obtained from rats with Flexner-Jobling carcinoma and from humans with gastric cancer hydrolized dl-leucyl peptides but failed to hydrolize d-leucyl peptides at any appreciable rate or to any significant extent. Attempts to produce d-peptidases artificially in rat and mouse serums by repeated injections of dl-leucylglycine were not successful. Extracts of several microorganisms, however, were found to possess marked d-peptidase activity.—H. J. C.

BRUES, A. M., and E. B. JACKSON. [Collis P. Huntington Memorial Hosp., Boston, Mass.] THE RESISTANCE OF TUMOR CELLS IN TISSUE CULTURE TO THE GROWTH-INHIBITING ACTION OF AMINES. Cancer Research, 1: 557-563. 1941.

Most alipatic amines (excluding diamines and amino acids) and the phosphoric ester of ethanolamine are inhibitory to growth of normal cells in culture in concentrations of about 10 millimoles per liter. The inhibitory effect shows a high degree of dependence upon pH of the medium in the case of most of these amines; in passing from pH 7 to pH 8, the effective concentration is reduced 90%.

The amines produce an intense vacuolation of the cytoplasm within a few hours. This process consists in segregation of large aqueous lacunae surrounded by a protoplasmic reticulum. Both inhibition and vacuolation are reversible upon removal of the amine.

Sarcoma and carcinoma are much less susceptible to inhibition by amines than are normal cells, and will grow readily alongside of wholly inhibited normal cells. Vacuolation does not occur in malignant cells even with concentration of amines high enough to cause cell death.

The possibility that amines may play a part in the physiological regulation of growth is discussed.—Authors' abstract.

ENTENMAN, C., F. W. LORENZ, and I. L. CHAIKOFF. [Med. Sch., Berkeley, and Coll. of Agric., Univ. of California, Davis, Calif.] THE ENDOCRINE CONTROL OF LIPID METABOLISM IN THE BIRD. III. J. Biol. Chem., 134: 495-504. 1940.

The intramuscular injection into immature chickens of estrogenic compounds (estrone, estradiol, estradiol benzoate, ethinyl estradiol, and stilbestrol) increased the concentration of the total fatty acids, phospholipdis and cholesterol in the blood, whereas testosterone, progesterone, and desoxycorticosterone acetate had no effect. Pronounced stimulation of oviduct growth was noted within 48 hours and the extent of increase of growth paralleled the increase in blood lipid levels.—H. J. C.

KENSLER, C. J., K. SUGIURA, N. F. YOUNG, C. R. HALTER, and C. P. RHOADS. PARTIAL PROTECTION OF RATS BY RIBOFLAVIN WITH CASEIN AGAINST LIVER CANCER CAUSED BY DIMETHYLAMINOAZOBENZENE. Science, 93:308-310. 1941.

The riboflavin levels in the livers of rats fed unpolished rice and carrots were significantly lower than in rats fed a stock laboratory diet. If butter yellow was mixed with the rice even less riboflavin was found in the livers. Tumors which resulted from the carcinogenic diet contained about 20% of the riboflavin in normal rat livers. If a yeast supplement (Fleischmann 20-40) was given, normal liver riboflavin levels were maintained and no cirrhosis or cancer resulted.

Riboflavin deficiency coincided with the susceptibility to the carcinogenic effect of butter yellow. However, feeding 5 mgm. daily of pure riboflavin was almost without protective effect. Accordingly, various supplements were tested with and without the addition of riboflavin. Nicotinic acid, corn oil, or casein had no protective effect when given alone. Nicotinic acid plus riboflavin (5 mgm. daily) gave a 50% decrease in cancer incidence. When casein was supplemented with riboflavin (200 μgm. daily) only 3% liver cancer resulted. Animals which received 15% of the diet as yeast showed no gross or microscopic evidence of liver damage at 200 days.

It appears that at least two factors are lacking in the basal diet of brown rice and carrot which renders rats susceptible to the carcinogenic effect of butter yellow. One of these factors is riboflavin and the other is provided in the "vitamin-free" alcohol extracted casein. Work on the manner in which casein exerts its protective effect is now in progress.—M. B.

LIEBOW, A. A., W. J. McFARLAND, and R. TENNANT. [Yale Univ. Sch. of Med., New Haven, Conn.] THE EFFECTS OF POTASSIUM DEFICIENCY ON TUMOR BEARING MICE. Yale J. Biol. & Med., 13:523-538. 1941.

The growth of tumor 15091A was significantly retarded in ABC albino mice which had been on a diet deficient in potassium for 3 weeks before inoculation. If the mice were deprived of potassium after the tumor had been allowed to grow, there was no significant retardation. Chemical analyses showed that the tumors of the control and potassium deficient animals contained an approximately equal proportion of potassium.

The experimental animals were demonstrated to satisfy three well established criteria of potassium deficiency: (1) failure to gain in weight, (2) decrease in potassium and increase in sodium of voluntary muscle, (3) necrosis and replacement by granulation tissue of cardiac muscle. New evidence of potassium deficiency was a remarkable increase in the weight of the kidneys associated with hypertrophy and proliferation of the epithelium of the tubules, focal increase in interstitial connective tissue associated with necrosis of isolated epithelial cells, and dilatation of the tubules.

Upon realimentation young animals resumed growth, the cardiac lesions tended to heal by scarring, the kidney approached its normal weight and appearance, and the chemical structure of the muscle was restored.—A. A. L.

URA, S. ÜBER SPALTUNG VON DIPEPTIDE DURCH CARCINOMSEREN. [HYDROLYSIS OF DIPEPTIDES BY CARCINOMA SERUM.] Gann, 35:38-40. 1941.

The hydrolysis of d,l-leucylglycine by cancerous (41 cases) and noncancerous (45 cases) serums was investigated. The serums and d,l-leucylglycine were incubated for 48 hours. Hydrolysis was followed by the method of Waldschmidt-Leitz and Mayer. The following results, expressed as percentage hydrolysis (average values) were obtained: normal serums, 41.9; maximum, 73.3, minimum, 13.4; noncancerous serums, 40.0; benign tumor serums, 40.8; gastric ulcer serums, 40.8; cancerous serums,

53.3. Higher values were obtained with serums from cancer patients showing generalized weakness. Hydrolysis values returned to normal when the chief seat of the malignancy was removed.—P. P. C.

VALASQUEZ, J., and P. ENGEL. [Veterinary Sch., Nat. Univ., Bogotá, Colombia] URINARY EXCRETION OF GONADOTROPINS IN CATTLE SUFFERING FROM CANCER. Endocrinology, 27:523-524. 1940.

The urine of 5 nonpregnant cows suffering from cancer localized in the region of the eye gave a positive Friedman reaction when 5 cc. were injected intravenously and 5 cc. subcutaneously into infantile female rabbits.—C. A. P.

von EULER, H., and B. SKARZYNSKI. [Biochem. Inst., Univ. of Stockholm] UEBER DIE SPALTUNG VON DIPEPTIDEN IN CARCINOMATÖSEN UND NICHT CARCINOMATÖSEN SEREN. [CLEAVAGE OF DIPEPTIDES BY CARCINOMATOUS AND NONCARCINOMATOUS SERUMS.] Ztschr. f. physiol. Chem., 265:133-146. 1940.

The cleavage of pure d-leucylglycine and of l-leucylglycine by human and animal blood serums was studied by means of titration of carboxyl groups, liberated in the course of the enzymatic process, in alcoholic solution according to Willstätter and Waldschmidt-Leitz. In confirmation of previous findings of Waldschmidt-Leitz and Mayer, an appreciable fission of the d-peptide by the serums of human cancer patients and of rabbits with Brown-Pearce sarcoma was observed, although not invariably. If the experiments are extended over long periods, some cleavage of d-peptide is occasionally observed in experiments with normal human serums.—K. G. S.

von EULER, B., and H. von EULER. [Biochem. Inst., Univ. of Stockholm] ZUR METHODIK DER CHEMISCHEN VITAMIN E-BESTIMMUNG. [TECHNIC OF CHEMICAL VITAMIN E ASSAY.] Ztschr. f. physiol. Chem., 265:147-151. 1940.

The content of vitamin E (α-tocopherol) in rat Jensen sarcoma was determined by the colorimetric method of Emmerie and Engel (Rec. Trav. Chim. Pays-Bas, **58**:895. 1939). The concentration found, 0.9 mgm. α-tocopherol in 100 gm. of fresh sarcoma tissue, freed from necrotic tissue, corresponds to that of normal tissues, rich in vitamin E.—K. G. S.

Immunology

BUNTING, H. [Yale Univ. Sch. of Med., New Haven, Conn.] STUDIES OF REGRESSION IN A TRANSPLANTABLE TUMOR IN MICE. Yale J. Biol. & Med., 13:513-522. 1941.

Regressions of tumor 15091A were most common in those adult mice in which there was an intermediate number of takes (Dba and C-Bagg albino), rather than in the most susceptible (ABC albino and ABC-AC), or in the most resistant animals (C57 BLK). When 1- or 2-day-old Dba or C-Bagg albino mice were inoculated with the tumor the proportion of takes was greater and the regressions less. Individuals in which the first graft had regressed were resistant to reinoculation. Such animals of the Dba strain in which the tumor failed to grow, however, were not resistant, but the contrary was observed with the C-Bagg albino strain. The early growth of ultimately regressing or progressing tumors was approximately the same, but then a lag in growth occurred in the former followed by the actual regression. Rarely was ulceration observed in the regressing tumors. Histologically, tumors removed 5 days after diminution in size had first been noted were almost completely necrotic. They were either sharply encapsulated or permeated by fibroblasts. In both types there was evidence of previous vascularization of the graft but the vessels now were filled with fibrin or agglutinated erythrocytes and lined by necrotic endothelial cells. Ultimately the tumor was replaced by scar tissue.

The complete necrosis of the regressing tumor despite previous vascularization, as well as other observations recorded here, suggested that regression was the result of the development of resistance by the host.—A. A. L.

OSWALD, W., and H. KUTTELWASCHER. [Inst. für allg. und exper. Path. der Univ. Wien, Austria] ZUR SEROLOGISCHEN ANTIGEN-ANALYSE DER IMPFTUMOREN II. [SEROLOGIC ANTIGEN ANALYSIS OF TRANSPLANTABLE NEOPLASMS II.] Ztschr. f. Immunitätsforsch., 98:427-430. 1940.

After flocculation of homologous and heterologous antiserums with appropriate amounts of extracts of normal intestinal tissue and ascites cancer of the mouse the supernatant fluids were tested for their complement binding power in the presence of each antiserum. After flocculation with anti-intestinal serum cancer extracts bound complement only in the presence of anticancer serum. The complement binding power of the supernatant resulting from a combination of anti-intestinal serum and intestinal extract was lost for both anticancer and anti-intestinal serums.—M. J. E.

SAPHIR, O., M. APPEL, and A. A. STRAUSS. [Michael Reese Hosp., Chicago, Ill.] GROWTH OF BROWN-PEARCE CARCINOMA IN THE ANTERIOR CHAMBER OF THE EYE OF TUMOR-IMMUNE RABBITS. Cancer Research, 1:545-547. 1941.

Immunization of rabbits to the Brown-Pearce carcinoma by intracutaneous transplantation does not confer protection against the growth of this tumor in the anterior chamber of the eye, despite the fact that all other tissues which were tested are resistant to its growth following such intracutaneous transplantation. However the growth of the tumor transplants in the eyes of such tumor-immune rabbits is considerably delayed.—Authors' summary.

Clinical and Pathological Reports

Diagnosis—General

ADAIR, F. L. [Univ. of Chicago, Med. Sch., Chicago, Ill.] HOW MAY THE GENERAL PRACTITIONER DIAGNOSE CANCER OF THE UTERUS? New England J. Med., 224:497-501. 1941.

This is a brief article stressing the importance of early diagnosis and presenting the salient features about the incidence and aids to diagnosis in carcinoma of the uterus.—A. M.

GRUNER, O. C. [Montreal, Canada] EXPERIENCES WITH THE PFEIFFER CRYSTALLIZATION METHOD FOR THE DIAGNOSIS OF CANCER. Canad. M. A. J., 43:99-106. 1940.

Encouraging results were obtained with the Pfeiffer cupric chloride crystallization test for cancer. To perform

this test 1 drop of blood is diluted with 1 cc. distilled water. Thirty minutes later 2 drops of this suspension are added to 10 cc. of 20% cupric chloride. The mixture is poured into a plate and the crystalline patterns formed are examined after 18 hours. Normal blood produces interweaving straight crystal bundles in distinction to the lines branching at 5 to 30 degrees resulting from cancer blood. Body fluids or tissues may be substituted for blood. A positive test was observed with 110 of 122 samples of blood from cancer patients and a negative reaction with 32 of 33 noncancer bloods. Of 20 tests with blood of patients cured of malignant disease or receiving radiotherapy only 9 proved positive. Photographs of crystals are reproduced.—M. J. E.

HELLWIG, C. A. [Sedgwick County Tumor Clinic, Wichita, Kan.] RAPID TISSUE DIAGNOSIS. Arch. Surg., 42:788-794. 1941.

In 4,326 biopsies, the microscopic diagnoses obtained from rapid tissue sections and from paraffin sections were in complete agreement in 95.4% of all cases. Malignancy or benignancy was correctly recognized in 96.9% of the cases by the rapid method. Noncarcinomatous lesions of the lymph nodes (Hodgkin's disease, lymphosarcoma, etc.) are the hardest tissues to diagnose by the supravital method, but carcinoma, metastatic in lymph nodes, is as easy to diagnose as the primary lesion. Terry's method and Hall's modification of his method of rapid tissue diagnosis are described. The author considers this technic superior to frozen sections.—G. De B.

WOLFSON, S. A., S. REZNICK, and L. GUNTHER. [Cedars of Lebanon Hosp., Los Angeles, Calif.] EARLY DIAGNOSIS OF MALIGNANT METASTASES TO THE SPINE. J. A. M. A., 116:1044-1048. 1941.

Early diagnosis of spinal metastases, in the absence of roentgenological evidence, may be made on the basis of the following triad of symptoms: (1) the presence of radicular pain, in narrow zones, sharply limited to one or two spinal nerve roots, associated with localized tenderness to deep percussion over the spinous processes of the vertebrae which form the foramens of exit for the nerve roots of the painful dermatomes; (2) an accelerated erythrocyte sedimentation rate; (3) an increase in the serum phosphatase level.—H. G. W.

WOODHOUSE, D. L. [Univ. of Birmingham, Birmingham, England] THE CHEMODIAGNOSIS OF MALIGNANCY. Am. J. Cancer, 40:359-374. 1940.

The author classifies the varieties of diagnostic reactions for cancer proposed to date into four groups. Group I includes tests involving analyses for specific chemical constituents of blood. Group II is made up of physicochemical determinations on blood and cells. Group III contains reactions of the antigen-antibody type and reactions dependent on enzyme changes. Group IV includes tests involving hormone assays.

After discussing the theoretical possibilities presented by the several groups, a report is given of experiments with three tests, (1) the lipolysis test; (2) the vanadate flocculation test of Cronin Lowe and (3) the Fuchs proteolysis reaction. The lipolysis test depends upon a decrease in the ability of serum from cancer patients to activate pancreatic "prolipase." In the vanadate flocculation test, a comparison of flocculations produced in the serums of healthy and cancerous persons by the addition of solutions of sodium ortho-vanadate is made. The theory underlying the Fuchs proteolysis reaction contends that the body produces an antibody to malignant tissues which alters reactions between serum, enzymes, and protein substrates.

Of the three methods, the Fuchs reaction apparently gave the most favorable results. However, its specificity is not such as to be of much aid at present. The author feels, however, that the results are encouraging enough to merit further work.—L. L. W.

Therapy—General

ADAIR, F. E. [Cornell Univ. Med. Sch., New York, N. Y.] INSTITUTIONAL CONVALESCENT CARE—THE CANCER PATIENT. Bull. Am. Coll. Surgeons, 26:46-47. 1941.

Prolonged radiation therapy, chronic ulceration, bone metastases, artificial stomata for feeding, etc., tend to make the convalescent care of the cancer patient a peculiar one, requiring special institutions.—A. M.

ALTER, N. M. [Jersey City, N. J.] LOW TEMPERATURE TREATMENT OF MALIGNANCY. J. M. Soc. New Jersey, 37:586. 1940.

A short account is given of the results of refrigeration in 27 cases of advanced cancer treated in Lenox Hill Hospital, New York, N. Y. The patients were maintained at a constant room temperature of 50° F. which reduced the rectal temperature to 90°. During or following treatment 13 died, most commonly of pneumonia. In a number temporary relief from pain resulted. Histologically no changes in the tumors were observed. Local refrigeration produces more conspicuous necrosis but offers no greater promise of being a permanent cure. Several leukemic patients were also treated, but no obvious improvement occurred.—M. J. E.

BRUNKOW, C. W. [Portland, Ore.] TUMORS IN INFANTS AND CHILDREN. CONSERVATIVE SURGERY THERAPY. West. J. Surg., 48:480-485. 1940.

Because of the high mortality following extensive surgery in children the author makes a plea for conservative measures, to be combined with radiotherapy when feasible. Only a relatively small number of patients tabulated by the author had neoplastic disease, the majority suffering from congenital malformations. Multiple stage operations and marsupialization are suggested devices to reduce surgical hazards.—M. J. E.

FAY, T. [Temple Univ. Med. Sch., Philadelphia, Penn.] OBSERVATIONS ON PROLONGED HUMAN REFRIGERATION. New York State J. Med., 40:1351-1354. 1940.

The author discusses reduction of body temperature as a therapeutic agent in a case of advanced cancer. Rectal temperatures as low as 80° F. may be maintained for several hours without deleterious effects. In many instances relief of pain obviated the need for further medication.—M. J. E.

JONES, A. J., J. GRAHAM, and A. MUELLER. [St. John's Hosp., Springfield, Ill.] LOCAL AND GENERAL TEMPERATURE REDUCTION IN MALIGNANCY. Am. J. Surg., 52:14-23. 1941.

Treatment of 14 cases of inoperable cancer with general or local hypothermia, with no evident prolongation of life, but a noteworthy relief of pain.—H. G. W.

McCRAVEY, A. [Temple Univ. Hosp. and Sch. of Med., Philadelphia, Penn.] THE TREATMENT OF TUMORS OF THE BLADDER BY REFRIGERATION. New York State J. Med., 40:1435-1440. 1940.

Local refrigeration by means of cold water circulated in the bladder at 40° F. for 43 to 133 days through a rubber or metal applicator was instituted in 4 patients with advanced vesical cancer. Surgery and irradiation had been attempted previously in 3 cases. Two patients died of ascending pyelonephritis. Extensive disintegration and complete regression of the tumors occurred in 2 patients and after the conclusion of therapy they appeared to be tumor-free locally.—M. J. E.

NAITO, K., M. SEGI, M. ITO, and M. MIYABE. [Frauenklin. der Kais. Univ. zu Tokyo] ÜBER DIE KOMBINATION VON TRAUBENZUCKER- und INSULIN-INJEKTION BEI DER STRAHLENBEHANDLUNG DER KREBSKRANKEN. [COMBINED GLUCOSE AND INSULIN TREATMENT ASSOCIATED WITH RADIOTHERAPY OF CANCER PATIENTS.] Monatschr. f. Krebsbekämpf., 9:32-32. 1941.

According to the authors cancers are made more radiosensitive if a hyperglycemia is maintained by means of oral or intravenous administration of glucose during irradiation. Following radiotherapy hypoglycemia produced by insulin protects the body against the deleterious effects resulting from the absorption of degenerative tumor products.—M. J. E.

PACK, G. T., and W. O. WUESTER. [Memorial Hosp., New York, N. Y.] THE TREATMENT OF MALIGNANT TUMORS OF THE SKIN. South. Surgeon, 9:775-796. 1940.

A review of the accepted surgical and radiotherapeutic methods employed in the treatment of skin cancers.—M. J. E.

ROFFO, A. H. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires] VOLUMINOSO PARAFINOMA OSIFICANTE DE LA RODILLA CURADO CON XILOL. [LARGE BONE-FORMING PARAFFINOMA OF THE KNEE CURED WITH XYLOL.] Bol. Inst. de med. exper. para el estud. y trat. de cáncer, 55:731-734. 1941.

Report of a patient who in order to simulate a tumor had paraffine injected in the knee. A large tumor developed which became ulcerated. After clearing the infection xylol dressings were applied for 1 month, and this brought about the total disappearance of the tumor by dissolving the paraffin. The histological examination showed a typical paraffinoma with formation of bone.—M. D-R.

SMITH, L. W. [Temple Univ. Med. Sch., Philadelphia, Penn.] REFRIGERATION IN CANCER. PATHOLOGIC OBSERVATIONS IN 100 ADVANCED CASES. New York State J. Med., 40:1355-1361. 1940.

The most impressive findings in treated cancers were regressive changes progressing to necrosis, diminution in size of tumors, and occasionally disappearance of local masses. Tumors reacting favorably were situated peripherally and amenable to local reduction in temperature to 40-50° F. General refrigeration with consequent reduction of the body temperature to 75-90° F. was applied in patients with deeper metastatic growths, but the favorable effects were less pronounced than in the previous group.—M. J. E.

STRODE, J. E., and E. A. FENNEL. [Honolulu, T. H.] THE TREATMENT OF TUMORS OF THE SHOULDER REGION BY INTERSCAPULOTHORACIC AMPUTATION. Surgery, 9:394-402. 1941.

This extensive crippling operation is reserved chiefly for relatively benign radioresistant tumors about the shoulders, which cannot be excised widely enough due to the nearness of structures important to one arm. Two successful cases are cited.—A. M.

SUNDERLAND, D. A., and J. C. BINKLEY. [Memorial Hosp., New York, N. Y.] THE USE OF ZINC PEROXIDE IN INFECTED TUMORS AND RADIATION NECROSIS. Radiology, 35:606-615. 1940.

A selected series of 52 patients treated with zinc peroxide is reported. They were divided into 3 groups: those with primary infected tumors; those with infected radiation necrosis; and the postoperative group in which the material was used either prophylactically or in already infected defects. The best results were obtained in the postoperative prophylactic group although zinc peroxide was of greatest use in the patients with infected radiation necrosis.—E. A. L.

TALBOTT, J. H. [Harvard Med. Sch., Boston, Mass.] THE PHYSIOLOGIC AND THERAPEUTIC EFFECTS OF HYPOTHERMIA. New England J. Med., 224:281-288. 1941.

The term "hypothermia" is preferred to "refrigeration" or "hibernation," etc. The author feels that the procedure has a place in the treatment of a selected group of patients with malignant disease. Relief of pain is the most important therapeutic gain. The article reviews the work of Smith and Fay, and others, as regards procedure, results, and hazards. The effects on the circulation, blood elements, chemistry, etc., are mentioned and the value of the treatment in other fields such as morphine addiction and schizophrenia is discussed. The author had 2 deaths in 20 cases of his series. A bibliography of 45 papers is given.—A. M.

VOEGTLIN, C. [Nat. Cancer Inst., Bethesda, Md.] POSSIBILITIES OF IMPROVED THERAPY FOR CANCER PATIENTS. J. Nat. Cancer Inst., 1:585-598. 1941.

This article reviews briefly the latest developments in the experimental field bearing upon cancer therapy. The therapeutic possibilities of neutrons, supervoltage x-rays, various forms of bacterial "toxins" and other chemical substances are discussed. The importance of lay and professional education in cancer is also stressed.—L. L. W.

VOEGTLIN, C. [Nat. Cancer Inst., Bethesda, Md.] POSSIBILITIES OF IMPROVED THERAPY FOR CANCER PATIENTS. J. A. M. A., 116:1491-1497. 1941.

A general review, the Barnard Hospital Lecture.—H. G. W.

WILSON, L. [Morrisania City Hosp., New York, N. Y.] ACTION OF TESTOSTERONE PROPIONATE IN A CASE OF ENDOMETRIOSIS. Endocrinology, 27:29-32. 1940.

A case report is given of a 28-year-old woman with endometriosis of the recto-vaginal septum. Testosterone propionate (50 mgm. per injection) was given every 2nd or 3rd day for 3 months and then every 3rd or 4th day for about 10 months (4,800 mgm. injected in 13 months). Typical masculinization occurred accompanied by suppression of menstruation for a year, an incomplete reduction of the size of the tumor, and complete freedom from pelvic pain. With cessation of treatment ovarian function returned promptly as also did the revival of growth of the tumor and return of pelvic pain.—C. A. P.

Skin and Subcutaneous Tissues

STECKER, J. F., and W. L. ROBINSON. [Univ. of Toronto, Toronto, Canada] DERMATOFIBROMA. Arch. Dermat. & Syph., 43:408-503. 1941.

Dermatofibroma is the name applied to a small, hard, fairly well circumscribed lesion originating as a benign growth in the corium of the skin and having certain features characteristic of a simple fibroma, yet being nonencapsulated, infiltrative, and slowly growing. Frequently it is diagnosed as sarcoma, but the clinical history proves it to be benign.—H. G. W.

STROUD, S. K., and C. D. STEWART. [Corpus Christi, Tex.] SOME EPITHELIAL TUMORS OF THE EYELIDS AND THEIR MANAGEMENT. Tex. State J. Med., 36:426-429. 1940.

A report of 3 cases of squamous cell cancer and 1 case of melanoma of the eyelids treated surgically.—M. J. E.

TORREY, F. A., and E. A. LEVIN. [Univ. of California Med. Sch., San Francisco, Calif.] COMPARISON OF THE CLINICAL AND THE PATHOLOGIC DIAGNOSES OF MALIGNANT CONDITIONS OF THE SKIN. Arch. Dermat. & Syph., 43:532-535. 1941.

Of 4,213 lesions, 2,806 were epitheliomas, representing 90% correct clinical diagnosis. There were 1,270 squamous cell and 1,519 basal cell epitheliomas, with a correct clinical differentiation in 75%. Fifteen % of the lesions diagnosed clinically as benign were found to be epitheliomas, and 37% of the lesions clinically diagnosed as keratoses were malignant. Seventy % of the epitheliomas occurred on the face, of which 37% were squamous cell and 63% were basal cell. Five % of the epitheliomas occurred on the dorsum of the hand or the exposed portion of the forearm, all of them being squamous cell. No basal cell epithelioma occurred on the mucous surfaces, and no squamous cell epithelioma occurred on the scalp.—H. G. W.

WOLFER, J. A. [Chicago, Ill.] TUMORS OF THE HEAD, FACE, AND NECK. Indust. Med., 10:59-60. 1941.

General remarks on diagnosis and treatment.—M. J. E.

Eye

DANDY, W. E. [Baltimore, Md.] RESULTS FOLLOWING THE TRANSCRANIAL OPERATIVE ATTACK ON ORBITAL TUMORS. Arch. Ophth., 25:191-216. 1941.

Orbital tumors frequently extend into the cranial cavity or form only a portion of a larger, primary intracranial mass. Intraorbital removal presents little possibility of a cure. A transcranial approach, as employed in resecting hypophyseal growths, may permit a complete resection or a successful palliative operation. Data are presented on 24 cases in which the latter operation was employed. The tumors produce exophthalmus, and an intracranial component may frequently be ascertained roentgenologically by changes in size of the optic foramen and hyperostosis of the walls of the skull and orbit. Meningioma, sarcoma, osteoma, and fibroma are the common neoplastic types, but Schüller-Christian xanthomatosis, cysts, or local inflammatory reactions may simulate neoplastic disease and demand similar treatment. Five patients died, 1 postoperatively and 4 after short intervals or as late as 10 years. Three appeared cured, but in the remainder, as a complete resection was not possible because of the large size of the mass or the danger of injuring vital intracranial centers, only a palliative result can be expected. Many of the tumor types recorded proliferate slowly and the patients survive a number of years. Details of operative technic are described and illustrated by drawings, and photographs of patients and roentgenograms are reproduced.—M. J. E.

IMRE, H. [Royal Hungarian Univ. of Budapest, Budapest, Hungary] DICTYOMA IN AN EARLY STAGE: REPORT OF A CASE. Arch. Ophth., 25:629-636. 1941.

Dictyoma is a rare malignant tumor of the ciliary epithelium containing cell bands resembling embryonic retina. It consists of two elements, solid cell masses of the type observed in glioma of the retina with scattered formation of rosettes, and distorted bands of cylindrical calls with an adenoma-like arrangement. The tumor in the author's case, approximately the dimensions of an adult lens, was found in an enucleated eye of a boy of 2 years. Its potentially malignant nature was demonstrated by the infiltration of the sclera.—M. J. E.

McKEE, S. H. [Montreal, Canada] MALIGNANT MELANOMA OF THE UVEAL TRACT: AN ANALYSIS OF FORTY-TWO CASES. Arch. Ophth., 25:238-242. 1941.

Of the 42 cases the tumor was situated in the choroid in 37, in the ciliary body in 3, and in the iris in 2. The visual disturbance following separation of the retina produced by a choroidal tumor is the common initial symptom of the disease. The possibility of a cure following enucleation is greatest when the tumor is the spindle cell type with intercellular argyrophil fibers. Ten patients died, 2 of complicating disease and 8 of metastases. Metastatic deposits occur at times many years following removal of the primary tumor.—M. J. E.

WELLER, C. V. [Univ. of Michigan, Ann Arbor, Mich.] THE INHERITANCE OF RETINOBLASTOMA AND ITS RELATIONSHIP TO PRACTICAL EUGENICS. Cancer Research, 1:517-535. 1941.

Although it is actually a very rare neoplasm (1 case in 34,000 living births), families in which retinoblastoma has appeared in one-half, or more, of the children have long been known. Only recently has it been appreciated that this disease may exhibit also a vertical familial distribution. Thirty families showing retinoblastoma in successive generations or in collateral lines have been collected. These show the transmission of the predisposition to retinoblastoma through both male and female, demonstrating and nondemonstrating, parents. Many examples of retinablastoma give the impression of being sporadic cases but even these have a possible relationship to the familial group. There is no clear-cut evidence that the inheritance of retinoblastoma is determined by either simple mendelian dominance or recessiveness. Rather, it appears that some structural anomaly, a somatic variation, is inherited and that upon this basis the neoplasm may develop. Relationships to tuberous sclerosis, glioma, and neurofibromatosis are recognized. Sterilization of any child surviving treatment for retinoblastoma and the interdiction of further children to the parents of a child with retinoblastoma appear to be justifiable measures.—Author's abstract.

Gastrointestinal Tract

COHEN, I. [New Orleans, La.] OBSERVATIONS ON MALIGNANCIES OF THE LARGE BOWEL. South. Surgeon, 10:173-184. 1941.

Delay in treatment, either because of an erroneous diagnosis or inattention by the patient to serious symptoms, accounts for a considerable percentage of failures in the treatment of cancer of the bowel. Recurrent bleeding, constipation, and abdominal distress always indicate the possibility of neoplastic disease, and careful roentgen studies and proctoscopic examination should be performed immediately. Four examples of a delayed diagnosis in patients with rectal cancer are recorded despite a clear symptomatology of the condition. When the true nature of the condition was established, a total extirpation was possible in only 1 case. Two of 3 patients with cancer of the transverse colon were given liver extract for a time under the impression that weakness and abdominal tympsoms were attributable to pernicious anemia. On the other hand, as an example of abdominal pain and intestinal bleeding from other sources a case of Banti's disease is cited. Roentgen examination in the latter case disclosed a normal colon.—M. J. E.

FELSEN, J. [Bronx Hosp., New York, N. Y.] THE DIFFERENTIAL DIAGNOSIS OF INTESTINAL POLYPOSIS. Am. J. Roentgenol., 45:551-557. 1941.

Adenomatosis of the colon is a neoplastic disease in which the mucosa of the large bowel from the rectum to the ileocaecal valve, is studied, with adenomatous tumors. The intervening mucosa is normal. The etiology is unknown. True malignancy is a frequent aftermath. Ideal treatment is complete colectomy.

Polyposis cystica of the intestine is an inflammatory disease, and is the late result of bacillary dysentery. Mucosal ulceration, fibrosis, and stenosis may involve the small as well as the large intestine. Malignancy never supervenes.—E. A. L.

LANDSMAN, A. A. [Jewish Memorial Hosp., New York, N. Y.] AMEBIC DYSENTERY AS A COMPLICATION IN THE DIAGNOSIS OF CARCINOMA OF THE RECTUM. New York State J. Med., 41:1181-1182. 1941.

A diagnosis of amebic dysentery was established in a woman of 52 years by isolation of the organisms in the stool. She improved under specific therapy, but the symptoms of gastrointestinal distress and rectal bleeding returned several months later. Sigmoidoscopic examination, however, now disclosed a carcinoma in the rectosigmoidal area with no evidence of recurrent amebic disease.—M. J. E.

MacCARTY, W. C., SR. [Mayo Clinic, Rochester, Minn.] EARLY CANCER OF THE STOMACH. A STUDY OF 1,299 RESECTED ULCERS AND 2,408 CANCERS. Cancer Research, 1:536-537. 1941.

This is a survey of statistics of cases observed by the author during the period of 23 years from 1918 to 1940. It presents yearly data on the sizes of resected gastric cancers and the degree of lymph nodal involvement. A gradual increase in early diagnosis was noted during the past 10 years. The author emphasized the need for greater provision for educating the practitioner and his patients to secure more frequent x-ray studies in all cases of vague abdominal distress.—S. B-J.

MacFARLANE, J. A. [Toronto Gen. Hosp., Toronto, Canada] CANCER OF THE RECTUM. Canad. M. A. J., 43:467-469. 1940.

The author contrasts the formerly hopeless attitude regarding surgical treatment of rectal cancer and the more rational modern opinion. From 1920-25 it was deemed advisable to attempt a complete tumor resection in only 6 of 57 cases observed, while in the period 1930-40 a radical resection was performed in 72 of 110 cases. In the remaining patients the growths were considered too advanced and a palliative colostomy was performed or no treatment attempted. The apparent higher percentage of operability is the consequence not only of earlier diagnosis, but of the development of improved operative procedures. Of the 72 patients in the second group subjected to a radical resection, 5 died postoperatively, 26 were without a recurrence 1 to 5 years after operation, and 16 appeared cured for longer periods.—M. J. E.

McNEER, G. [New York, N. Y.] CANCER OF THE STOMACH IN THE YOUNG. Am. J. Roentgenol., 45:537-550. 1941.

Five hundred and one cases of gastric carcinoma in patients under 31 years of age are accepted for statistical analysis from the world literature and compared with 682 cases from the Memorial Hospital in all age groups. The symptomatology of the disease in the young closely resembles that in the older subjects. However, loss of weight was a complaint in only 21.6% of the young as compared to 85% in the older patients; and, whereas vomiting was an outstanding factor in 64.3% of the young and pain in 62.2%, the order was reversed in the older patients with pain in 69.6% and vomiting in 44.7%. Cachexia was a much more prominent feature in the older group than in the young. Other outstanding features of the disease in the young were: a 27.3% resectability compared to 9.3% in the older group; and a high incidence of Krukenberg tumors and of pulmonary metastases. Of the total 501 patients 6.4% survived the operation for 3 years, and 3.8% lived 5 years. In the group of older patients 1.1% of the total survived 3 years and 0.7% lived five years.—E. A. L.

MORRISON, W. B. [Columbus, Ohio] CURABILITY OF CANCER OF THE STOMACH. Ohio State M. J., 37:453-456. 1941.

An analysis is given of 214 cases of gastric cancer observed between 1930 and 1940. A resection was performed in 76 cases, a palliative operation in 60, and in 78 the disease was considered too advanced to justify intervention. Although the average operative mortality was high (28%), it appeared to be declining in more recent years as a result of technical improvements and earlier diagnoses. The ultimate prognosis is poor. Only 8 of the 76 patients who underwent a radical surgical intervention survived 5 years or more after operation. One patient died of cancer after 6 years.—M. J. E.

RIVER, L., R. W. McNEALY, and A. B. RAGINS. [Cook County Hosp., Chicago, Ill.] CARCINOMA OF THE AMPULLA OF VATER. Am. J. Surg., 52:289-296. 1941.

Three instances of transduodenal resection of periampullary carcinoma are recorded, successful from the surgical standpoint, although 1 patient died from meta-

stases, and the survival period in the other 2 was short.—H. G. W.

WILLIS, R. A. [Univ. of Melbourne, Victoria, Australia.] ARGENTAFFIN CARCINOMATA ("CARCINOIDS") OF THE SMALL INTESTINE. Med. J. Australia, 2:400-404. 1940.

Six post-mortem cases of argentaffin carcinoma of the small intestine are described. Metastases were present in 4 cases, and in 2 the deposits were multiple, remote, and blood-borne. "These findings denote a high degree of malignancy and show the falsity of the implication of innocence in Oberndorfer's name 'carcinoid' tumour; the tumours are frank carcinomata, sometimes extremely malignant carcinomata." Various diagnostic difficulties, both pathological and clinical, are also discussed.—A. H.

YARDUMIAN, K. Y., and I. B. SWICKLEY. [Montefiore Hosp., Pittsburgh, Penn.] PRIMARY NONEPITHELIAL TUMORS OF THE STOMACH. Am. J. Surg., 52:346-354. 1941.

Of 122 cases of primary tumors of the stomach, 5 were nonepithelial, of which 3 are living and well after surgical removal.—H. G. W.

STATISTICS

BARGEN, J. A., C. W. MAYO, and L. A. GIFFIN. [Mayo Clinic, Rochester, Minn.] FAMILIAL TRENDS IN HUMAN CANCER. J. Hered., 32:7-10. 1941.

When multiple cancers occur in any family and one cancer at least is localized in the colon or rectum, there is a greater chance that cancers in other members of the family will be localized in these regions and also in the stomach than in cancerous families in which colonic and rectal cancer is not known. The transmission of cancer in humans is complex because of the many factors concerned in the relationships of human families.—J. J. B.

CRUICKSHANK, D. B. [Sims Woodhead Memorial Lab., Papworth, England] THE TOPOGRAPHY OF THE RELATIVE DISTRIBUTION OF CANCER AND TUBERCULOSIS. Tubercle, 21:281-291. 1940.

Statistical evaluation of the mortality rates for cancer and tuberculosis in England and Wales reveals a constant relative distribution of the two diseases. These facts are illustrated by appropriate tables and charts.—M. J. E.

HARTZ, P. H. [Pub. Health Dept., Curacao, N. W. I.] THE INCIDENCE OF MALIGNANT TUMORS IN UNSELECTED AUTOPSY MATERIAL AT CURACAO, NETHERLANDS WEST INDIES. Am. J. Cancer, 40:355-358. 1940.

In 650 autopsies on colored Curacao natives, 71 malignant tumors were found. The average age of those persons showing malignant tumors was 51.5 years. The material analyzed showed a preponderance of cancers of the esophagus and stomach, while rectal and colonic cases were rare.—L. L. W.

JACOBS, L. G. [Winona, Minn.] THE EVALUATION OF CANCER STATISTICS BY CORRELATION ANALYSIS. Radiology, 36:334-342. 1941.

Various difficulties in evaluating the numerous conflicting reports advocating the use of various forms of therapy in cancer are pointed out. The use of correlation analysis is recommended. The method is briefly reviewed, and an example is presented.—E. A. L.

KÖRBLER, J. [Staat. Radium-Inst., Zagreb, Yugoslavia] DAS KREBSGEFÄHRDERTE LEBENSALTER. [THE DANGEROUS AGE FOR CANCER.] Monatschr. f. Krebsbekämpf., 9:33-37. 1941.

The most common age of onset of illness of 2,377 cancer patients was between 50 and 60 years. The greatest frequency of mammary cancer occurred between 45 and 50 years. In estimating the frequency of disease proportional to the number living in the general population there was a tendency to displacement of the most frequent groupings to the higher ages. Nevertheless, despite this correction in statistical analyses, cancer appeared to be relatively uncommon in the most advanced age groups.—M. J. E.

LACROIX, L. [Royal Univ., Pisa] I BLASTOMI EPITELIALI MALIGNI NELLA PUERIZIA, NELL 'INFANZIA E NELLA GIOVENEZZA. (DELLA NASCITA FINO A TRENT'ANNI. [MALIGNANT EPITHELIAL BLASTOMAS IN INFANCY, CHILDHOOD AND YOUTH, FROM BIRTH TILL 30 YEARS OF AGE.] Pathologica, 32:61, 119, 159. 1940.

This is a statistical study based on 229,275 cases of epithelial cancer of all ages and on 6,193 cases of the same neoplasms in patients under 30 years of age. The latter form 2.7% of the total. The exact localization of the tumor could be determined in only 3,783 cases distributed as follows: digestive tract, 60%; female genital tract, 23%; mammary gland, 4.25%; male genital tract, 3.65%; urinary tract, 3.12%; skin, 2.77%; respiratory tract, 4.65%; endocrine organs, 0.98%; visual organs, 0.31%; circulatory system, 0.13%; nervous system, 0.10%; blood-forming organs, 0.05%. About 650 references are appended.—M. D-R.

LARSON, C. P. [Western State Hosp., Fort Steilacoom, Wash.] INTRACRANIAL TUMORS IN MENTAL HOSPITAL PATIENTS. A STATISTICAL STUDY. Am. J. Psychiat., 97: 49-58. 1940.

During a period of 2 years ending Oct. 1, 1938, 30 examples of intracranial tumor (in the broad sense of a localized swelling) were found in routine autopsies on 223 patients dying in a mental institution. Only 18 lesions were neoplastic. There were 4 cases of glioma, 4 of pituitary tumor, 8 of meningioma, and 1 case each of dural metastasis of a cancer of the breast and rectum. In the remaining cases the process was a chronic infection, cyst, or aneurysm.—M. J. E.

NEUMANN, A. [Vienna, Germany] SI LA ENFERMEDAD DE CANCER ES REALMENTE UNA ENFERMEDAD DE LA VEJEZ? [IS CANCER REALLY A DISEASE OF OLD AGE?] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 55: 871-879. 1941.

The material is mostly supplied by the statistics of the city of Buenos Aires published by Roffo. The relative incidence of sarcoma and carcinoma is studied and the conclusion is reached that although there is an increase of the former with advancing age it is much smaller if compared to the increase of carcinoma (7 times and 1,000 times respectively). Nevertheless, although the cases of sarcoma are only 5.6% of the 12,002 cases here studied, the economic loss is 11.3% of the total loss of productive years for the nation.—M. D-R.

PELLER, S. [Graduate Sch., New York Univ., New York, N. Y.] MALIGNANT MELANOMA CUTIS. Cancer Research, 1:538-542. 1941.

Out of 1,047 patients with cancer of the skin and lip, 4.1% had malignant melanoma cutis. In the civil popula-

tion, females suffering from surface cancer have a higher ratio of melanoma than males; both women and men have a lower ratio of melanomas than observed among soldiers and sailors. On the average, patients with melanoma are younger than other patients with cancer of the skin or cancer in general. The ratio of melanoma to all surface malignancies drops with advancing age. The age specific incidence rates of melanoma rise more slowly than those of the other malignancies. With regard to the incidence of melanoma the geographical or climatic factor plays a role different from that in the incidence of surface epitheliomas and of internal cancers. The distribution of melanoma on the surface of the body differs materially from that of epithelioma. On the head, of all surface malignancies, including lips, 0.72% were melanomas. Excluding the lower lip, the ratio rises from 0.72 to 1.0%. On the other parts of the skin the corresponding value is 16.2%, and in sailors and soldiers 34.6%. These facts indicate that for the change of a nevus into a melanoma, and for the higher incidence of melanoma in the armed forces other factors are responsible than those related to the pathogenesis of epithelioma of the skin and lip. With regard to melanoma the ultraviolet portion of the spectrum does not play a major role.—Author's summary.

PHILLIPS, C. [Scott and White Clinic, Temple, Tex.] THE RELATIONSHIP BETWEEN SKIN CANCER AND OCCUPATION IN TEXAS: A REVIEW OF 1569 VERIFIED LESIONS OCCURRING IN 1190 PATIENTS. Texas State J. Med., 36:613-616. 1941.

The incidence of cancer of the skin is known to be high in Texas. The author analyses, according to occupation, the occurrence of 1,569 verified skin cancers in 1,190 patients observed between 1920 and 1940. The subjects represent all walks of life and are not from a selected group. Only 1 Negro is included. Although a correlation of the number of patients with the total in the respective population groups was not made, there appears to be a higher incidence of skin tumors in the farmer-rancher group and their wives. These individuals furnished 46% of the skin neoplasia. The occurrence of melanoma was not related to the occupation of the tumor-bearers.—M. J. E.

WELLING, W. C. [State Dept. of Health, Hartford, Conn.] CONNECTICUT MORTALITY IN 1939. Connecticut M. J., 4:212-213. 1940.

In 1939 there was a decrease of 197 in the number of deaths charged to cancer as compared with 1938, but according to the author, analysis reveals the fact that this figure should be viewed with caution. In the records of the ten leading causes of death, cancer has risen from 7th place in 1910 and 1920 to 2nd place in 1938.—A. DeB.

CANCER CONTROL AND PUBLIC HEALTH

AMERICAN COLLEGE OF SURGEONS. [Chicago, Ill.] Bull. Am. Coll. Surgeons, 25:167-171. 1940.

The College is encouraging the establishment of organized cancer clinics in general hospitals of 100 beds or more. A "Minimum Standard for Cancer Clinics" serves as a guide. Of 1656 investigated institutions, 20% have been approved. Fifty per cent will bring approved facilities within the reach of all.

The College has accumulated over 30,000 records of 5-year survivals.

The Registry of Bone Sarcoma, established in 1921, contains 2,232 cases. These have been extensively analyzed and are largely responsible for the last revision of the bone tumor classification in 1939. The Registry has collected an extensive exhibit.—A. M.

DREYFUSS, M. [Elmira, N. Y.] THE ELMIRA TUMOR CLINIC. A SURVEY OF THE WORK AFTER SIX YEARS OF ITS EXISTENCE. New York State J. Med., 40:1725-1728. 1940.

This is a general description of the organization of a diagnostic tumor clinic in a smaller city. Of the 537 patients examined during a period of 6 years, 130 had malignant tumors. Benign growths, and a considerable number of nonneoplastic conditions such as chronic dermatologic lesions, cystic mastitis, and cervical polyp were found in the remaining group.—M. J. E.

INSTITUTIONS CONDUCTING CANCER CLINICS WHICH ARE APPROVED BY THE COLLEGE. Bull. Am. Coll. Surgeons, 25:661-669. 1940.

There are 345 approved cancer clinics. Eighty-one additional clinics are not quite ready for rating, and 64 other groups have signified their intention to form clinics soon. Only 5 states have cancer hospitals, of which there are 14. The "Minimum Standard for Cancer Clinics" is given. The approved cancer hospitals, cancer clinics, and cancer diagnostic clinics are listed.—A. M.

NATIONAL CANCER RESEARCH. New England J. Med., 224:296. 1941.

Grants-in-aid totalling $271,277.50, awarded by the National Advisory Cancer Council, are commented upon in this editorial. A list of groups of these grants is included. —A. M.

PACK, G. F. [Memorial Hospital, New York, N. Y.] THE CANCER RECORD FORMS OF THE AMERICAN COLLEGE OF SURGEONS. Bull. Am. Coll. Surgeons, 25:172-175. 1941.

The revised record forms adopted by the National Advisory Cancer Council are described and their value discussed. A sample "synoptic cancer record form" for rectal tumor is shown.—A. M.

SCIENTIFIC SOCIETIES AND RESEARCH ORGANIZATIONS

FOURTEENTH MEETING OF THE ASSOCIATION OF CONNECTICUT TUMOR CLINICS. Connecticut M. J., 4:142-147. 1940.

The following subjects were discussed: routine sterilization of patients with carcinoma of the breast, late metastases of melanoma, irradiation therapy of cancer of the cervix, and radical therapy of hydatid mole.—A. DeB.

Book Reviews

BIOLOGY OF THE LABORATORY MOUSE. By the Staff of the Roscoe B. Jackson Memorial Laboratory; edited by George D. Snell. The Blakiston Co., Philadelphia, 1941. 497 pages; 172 illustrations. Price $7.00.

Eight members of the staff of the Roscoe B. Jackson Memorial Laboratory and J. H. Dingle of the Harvard Medical School contributed one or more chapters on varying phases of the Biology.

The object has been to bring together from the published literature and from the observations of the authors the scattered information on the mouse. Since "not less than one million mice are raised each year in this country for research in bacteriology, cancer and genetics," the mouse must represent the most common laboratory animal. The subjects considered are essentially: 1. Morphology—embryology and histology. 2. Genetics—with special emphasis on cancer. 3. Oncology—morphological and other factors involved in spontaneous tumors. 4. Parasitology and infectious diseases.

The first chapter on the development of the fertilized ovum and embryo is extremely well organized and admirably illustrated, largely by original drawings and photographs. The implantation of the ovum, formation of the placenta and extra embryonic tissues are presented concisely. The development of the fetus is not described. The section devoted to reproduction is detailed and presents tabular data on the duration of pregnancy and size of litters in mice of some strains, as well as a discussion of ovogenesis, the estrous cycle, fertilization, pregnancy, and lactation. The greater part of the experimental studies on the physiology of reproduction were omitted although citations to general references on this subject were listed.

The histology of the organs and glands are discussed at length and will be of interest to students of comparative histology. The genital tissues are described most extensively. The morphological characteristics of spontaneous neoplasms are described in detail, especially the mammary tumors. The detailed structure of most of the tumors are shown in well chosen photomicrographs. This section should be of particular interest and value to investigations of cancer in mice. In some instances the tendencies of certain types of tumors to occur in mice of specific strains is mentioned. Reference has been made to many spontaneous tumors described in the literature.

The genetic studies of spontaneous mammary, pulmonary and nonepithelial tissues are summarized. Mammary tumors are the most frequent type of neoplasia encountered in unselected mice and the incidence of such tumors may be greatly increased by selection although, at the present time, extra-chromosomal factors, or factors transferred through the milk, have been demonstrated. The genetic studies on pulmonary tumors indicate a complex chromosomal transmission possibility of a dominant nature. The nonepithelial tumors with the exception of lymphoid tumors are not sufficiently common to permit an adequate genetic or extra-chromosomal interpretation. Tabular data have been selected to illustrate and summarize the presentation. The genetic studies on transplanted mammary and lymphoid tumors have been likewise concisely reviewed with selected tabular data and diagrams. The sites and technics of transplantation are mentioned and some of the transplanted tumors available in mice at several laboratories are listed.

The technics used in the development of inbred strains of mice are explained in another chapter. The phenotypic variations observed in inbred strains are accounted for as variable responses to the "physical environment" with or without genotypic determination of this susceptibility as well as the more commonly observed increased homogeneity of inbred strains. The values of hybrid mice of the first generation is properly emphasized. In some ways such animals are more suitable than their parents since they are equally uniform in most respects and usually more prolific and vigorous. Of greater interest to the pure geneticist is a detailed listing of known mutations and when possible their linkage groups.

The work on the effect of hormones and nursing influence or "milk factor" on mammary tumors is briefly reviewed.

Two especially valuable sections for all interested in experimental work with mice are on parasites (protozoa, helminths and arthropods) and infectious diseases (bacterial, fungus and virus). Descriptions are sufficiently detailed to permit identifications or diagnosis. Methods of treatment, because of the small size of the mouse, are uniformly preventive and involve maintaining mice in quarters free from wild mice and intermediary hosts, isolation of sick animals, maintenance in clean quarters, and on a good nutritional plane. The system of quartering, feeding and marking animals at the Jackson Memorial Laboratory are described in detail.

The material presented should prove to be valuable as a reference in laboratories engaged in studies of cancer or genetics in mice. Since controversial material is largely omitted and possible phases for investigation are mentioned it will be of special value to the reader familiarizing himself with the mouse as a laboratory animal. The absence of a chapter on nutrition and metabolism in mice probably indicates the lack of extensive studies in these fields.

W. U. Gardner.

THE BIOLOGIC FUNDAMENTALS OF RADIATION THERAPY. A Texbook by Friedrich Ellinger, M. D., with a Preface by Maurice Lenz, M. D. English Translation by Reuben Gross, M. D. Elsevier Publishing Company, Inc., New York, 1941. Distributors: Nordeman Publishing Co., 215 Fourth Ave., New York, N. Y. xvi + 360 CP. Price $5.00.

This textbook of radiation therapy contains 294 pages of text and 47 pages of bibliography. The book is an English translation of the previous German edition published in 1935. It has been largely revised and re-edited

as indicated by the bibliography of 1100 references representing contributions published during the last decade to April 1940. Part I, of 117 pages, deals with the effects of roentgen rays and gamma rays of radium on normal tissues of the body as well as effects of these radiations on infections, and benign and malignant neoplasms. Careful attention has been given to historical considerations in the presentation of important experimental work. Controversial data have been presented with references designed to stimulate the reader to consult original source material.

Part II of the book devotes 13 pages to a consideration of cathode rays and corpuscular radiation of radioactive bodies, with a brief note concerning neutrons, and artificial radioactive substances.

Parts III and IV of the volume deal with the biological effects of ultra-violet light, the action of visible and infra-red rays and together constitute 109 pages.

Part V, consisting of 33 pages, is devoted to general radiation biology and therapy with a concise discussion of radiosensitivity, the time factor in radiation therapy, and general principles governing clinical dosage.

The amount of space devoted to ultra-violet light and its biological effects is disproportionately large in comparison with the space allotment on the effects of roentgen and radium radiation on malignant neoplasms.

The book can be recommended as a text as well as an excellent reference book of recent contributions in the field of radiation biology and biophysics.

H. M. Wilson.